AN *IN-VITRO* STUDY EVALUATING THE EFFICACY OF THE ULTRASONIC BYPASS SYSTEM™, USING DIFFERENT INTRACANAL IRRIGATING SOLUTIONS

by

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INTRODUCTION

The goal in endodontic therapy is to efficiently clean the root canal system through both mechanical and chemical means, leaving the root canal system as close to sterile as possible.[1-4] Accomplishing this goal increases the long-term success of endodontic therapy and improves the overall health of the patient.[5, 6] However, there are many obstacles that hinder the success of root canal therapy, such as the complex root canal system anatomy with all the fins, lateral canals, and connections, as well as the creation of a smear layer with its accumulation of inorganic and organic material all of which harbor bacteria. Most endodontic failures can be traced to persistent bacterial infection often times caused by inadequate root canal debridement.[7]

The smear layer is formed during mechanical cleansing of the root canal walls. Due to its inorganic and organic nature, this complex layer is difficult to remove with just one type of irrigating solution, thus requiring different irrigating solutions with varying properties that thoroughly remove it.[8, 9] Furthermore, Ram et al. showed that irrigating solutions only progress 1 mm further than the apical extent of the syringe needle from which it is expressed.[10] This presents a problem with canal curvature, fins, and irregular root canal walls, where penetration of the irrigating solution into these irregularities is needed for thorough debridement. With the advent of ultrasonics, however, irrigating solutions have been proven to penetrate further apically and have better success of removing the smear layer, cleaning excess debris, which accumulates with the fins and anatomizes, and reducing bacteria within the root canal system.[11-15]

The Ultrasonic Bypass System™ functions by expressing a constant flow of irrigating solutions in conjunction with ultrasonic vibrations. Both of which can greatly cleanse the smear layer from the canal walls. One mechanism by which this device functions is cavitation, which occurs at the apical 1 mm of the ultrasonic tip and with the device running at full capacity. The other mechanism by which canal wall debridement occurs is acoustic streaming, which occurs on the sides of the ultrasonic file and can be accomplished with either high or low energies.[16, 17] Of interest in this study is the examination of the Ultrasonic Bypass system with the common irrigation solutions often used in chemo/mechanical debridement of root canals, such as 6.0-percent sodium hypochlorite and 17-percent EDTA, and to study the effectiveness of smear layer removal. If the findings of this study are significant, clinicians can implement the use of a specific irrigation regimen to improve smear layer removal.

PURPOSE

The purpose of this study is to compare the debridement efficacy of different irrigating solutions using the Ultrasonic Bypass System™ (Vista Dental, Racine, WI) after rotary instrumentation in extracted human teeth. Using a scoring system and SEM, the amount of smear layer removal following the use of these different irrigating solutions will be evaluated.

HYPOTHESIS

Hypothesis *Ho*) No statistically significant difference exists between the mean scores of debris and smear layer removal by the four different irrigating-solution protocols, using the Ultrasonic Bypass System after hand-rotary instrumentation.

Hypothesis *Ha*) A statistically significant difference exists between the mean scores of debris and smear layer removal by the four different irrigating-solution protocols, using the Ultrasonic Bypass System after hand-rotary instrumentation.

REVIEW OF LITERATURE

HISTORY OF ENDODONTICS

As is the case with many aspects of life, knowing the future or direction of endodontics hinges on knowing where we have been. Endodontics, as Gutmann states "is the branch of dentistry concerned with the morphology, physiology, and pathology of the human dental pulp and periapical tissues."[1] Although early pioneers in endodontics may not have recognized this subdivision in dentistry, they truly were intrigued and spent considerable time studying the dental pulp.[18-21]

Humans have an aversion to pain. Much of our evolution has been influenced by our ability to avoid or minimize pain. One can only imagine the trials that early humans experienced as they dealt with the trauma and pain of a toothache. Experiences of the first US president, George Washington, exemplify the pain and affliction diseased teeth may cause. President Washington battled significant tooth pain, only to eventually succumb to it by wearing dentures. During the Revolutionary War, Washington requested the services of a dentist because of tooth and gum pain that the general wanted "relieved by a man of skill." Even some of Washington's portraits reveal his terrible state of tooth affairs. A painting by Charles Willson Peale of Washington at age 44 shows a scar on his left cheek from an abscessed tooth.[22, 23]

This problem of "toothache pain" and the fear of losing one's teeth not only plagued George Washington but have plagued the human experience for generations. Our literature, oral history, and art have shown this fear vividly. Miguel Cervantes, in his masterpiece *Don Quixote*, described the importance of keeping one's teeth when he wrote

"a mouth without teeth is like a mill without its stone and you must value a tooth more than a diamond."[24] The pain and fear of a toothache spurred early civilizations to relieve these toothaches. Chinese civilizations theorized dental disease to be the cause of "tooth worms" and show some of the early evidence of attempts to treat it. Evidence has been found in an early Nabatean skull about 2200 years ago, wherein a lateral incisor has been obturated with a bronze wire.[25]

ENDODONTIC THEORY

A classic article by Kakehashi et al.[5] highlighted the harmful effects microorganisms have in endodontic lesions. They used germ-free and non-germ free rats to study the effects of bacteria on pulpal disease and made surgical exposures on the teeth of both sets of rats and then left them open to the oral environment. At different time intervals the rats were sacrificed and histological examination was performed. In the germ-free rats, the surgical exposures showed no signs of pathosis or abscesses, only mild inflammation. At 14 days, dentinal bridge formation could also be detected on these rats. However, on the conventional rats, pulpal necrosis, abscess formation, and purulence were seen. From these findings, the authors concluded the presence or absence of a microbial flora determines the healing of exposed rodent pulps.

Early studies attempted to examine further the role of microorganisms on endodontic infections. These attempts were carried out with the use of histological exam or bacteriologic sampling. Reeves et al.[26] examined cariously exposed pulps with histological methods and found that bacteria indeed penetrated the pulp space.

Bacteriologic sampling during endodontic therapy was another way practitioners attempted to correlate microorganisms with endodontic infections. In treating teeth with periapical lesions, Brown et al.[27] examined teeth with unexposed pulp canals, no deep carious lesions, no discernible gross anatomic defects, no large restorations, no extensive periodontal involvement, and no history of trauma for the presence of bacteria. Through microbiological sampling, the authors found microbial forms in 90 percent of the specimens.

Matusow[28] determined the possible role specific bacteria might have on acute pulpal-periapical cellulitis by sampling seventy-six teeth during endodontic therapy. One hundred and eight microbes were isolated with streptococci representing 62 percent of the total microbes. Fifty-one percent were aerobic and 10 percent were anaerobic. Matusow concluded that streptococci were associated significantly with acute odontogeic infection. In another microbiological sampling study, Yoshida et al.,[29] using similar methods, found *Peptococcus magnus* and Bacteroides species associated with clinically acute cases and oral streptococci and enteric bacteria frequently isolated from asymptomatic cases. Griffee et al.[30] also cultured bacteria to correlate clinical symptoms in 33 cariously and traumatically exposed teeth. Cultures were taken during endodontic therapy and analyzed. *Bacteroides melaninogenicus* was statistically associated with pain, sinus tract formation, and foul odor. In more culturing studies Baumgartner et al.[31] cultured of the apical 5 mm of infected root canals and found fifty strains of bacteria from 10 root canals with 68 percent being strict anaerobes. The authors found the presence of predominately anaerobic bacteria in the apical 5 mm of infected root canals in teeth with carious pulpal exposures and periapical lesions.

Schein et al.[32] aspirated fluid from 40 endodontically involved teeth to evaluate endotoxin within the samples. Endotoxin is a lipopolysaccharide complex found in the cell walls of gram-negative bacteria that when released during endodontic infections act as potent biologic agents by activating the complement system through antigen-antibody complexes, by complement system activation directly by the endotoxin, or by the endotoxin inducing a dramatic, acute inflammatory response. Schein et al. found that teeth without pulps had greater concentrations of endotoxin compared to vital teeth. Also more endotoxin was collected from symptomatic teeth than asymptomatic teeth.[33] Dwyer et al. further showed the potent inflammatory role endotoxin plays in periapical lesions. The authors placed non-detoxified and detoxified endotoxin in root canals of cats. After two weeks radiographically the periapical tissues of the teeth with non-detoxified endotoxin showed breakdown and this continued up to six weeks. There were not any radiographic changes of the periapical tissues of the teeth with detoxified endotoxin sealed in their root canal systems. Histologically the periapical tissues were examined and those with the non-detoxified endotoxin showed a greater amount of inflammatory infiltrate. From these results, the authors summarized that the "radiographic and histologic results indirectly suggest that endotoxins have a part in initiating and perpetuating periapical inflammatory lesions in man."

With the advent of polymerase chain reaction (PCR), evaluation of endodontic lesions has greatly changed. Using DNA probes, PCR allows the clinician to evaluate with higher specificity the different microorganisms found within teeth with periapical lesions. Siqueira et al.,[34, 35] using PCR, evaluated teeth with endodontic lesions and found a greater diversity of microflora than previously evaluated. The authors stated

molecular methods revealed "a higher complexity" of the endodontic microbiota than previously shown and that in addition to detecting some cultivable species in increased prevalence, molecular methods expanded the list of putative endodontic pathogens "by inclusion of some fastidious bacterial species or even uncultivated bacteria that have never been previously found in endodontic infections."

Many studies have evaluated microorganisms and their role in endodontic infections. These studies show that endodontic lesions are polymicrobial in nature with anaerobes being the primary group.[36-41] Sundqvist[42] investigated through bacteriological sampling the correlation between the composition of microflora in teeth with apical periodontitis. In 32 traumatized human teeth, the author found apical periodontitis only occurred with teeth that had bacteria present within the root canal. As the number of species of bacteria increased so did the patients' symptoms. Also, the size of the periapical lesion was often found to be larger when six or more different species were present. However, teeth that were categorized as sterile necrosis, were free of periapical lesions.

In another animal study, Möller et al.[28] found similar results as Kakehashi et al. Apical periodontitis only occurred in monkeys with oral bacteria present and indigenous oral bacteria can survive in the root canal of these infected teeth. Again using monkeys, these authors found that once teeth were infected and then closed from the oral environment, the relative proportion of obligate anaerobes increased with time highlighting the selective mechanisms which allow certain bacteria to survive and proliferate. They also found that root canal infections were polymicrobial and excited as a complex and integrated group of bacteria that symbiotically existed together. [40, 41, 43]

BIOFILMS

Recently the concept of biofilms within the oral cavity has received considerable attention. Much has been written and researched about biofilms as they pertain within periodontal disease but little has been written about biofilms within the endodontic lesion.[44]

A biofilm is a "thin-layered condensation of microbes (e.g. bacteria, fungi, protozoa) that may occur on various surface structures in nature" (biofilm article). The three stages of biofilm formation as mentioned by Svensäter et al.[45] are:

1) Adsorption of macromolecules (proteins, glycoproteins) to a surface, leading to the formation of a conditioning film.
2) Adhesion and co-adhesion of planktonic bacteria
3) Multiplication and metabolism of attached microorganisms that ultimately result in a well organized mixed microbial community.
4) Detachment of microorganisms via enzymes from biofilm implicating possible biofilm spreading and colonization.

As biofilm forms, the physical makeup becomes crucial to its survival. Open channels are created wherein fluid flows carrying essential nutrients and waste, all of which helps maintain this biological diverse entity. Ultimately, the biofilm allows for microorganisms to survive. Another important benefit of a biofilm is, as a whole, bacteria are able to degrade large macromolecules and use for nutrients that as individual bacteria they may not be able to degrade. This is done by some bacteria expressing enzymes that other bacteria may not yet possess, but as these enzymes break down

proteins, nutrients then are available to the whole group including those who individually may not have been able to utilize these proteins.

Another important feature of biofilms is quorum sensing, a way in which bacteria communicate within a biofilm via diffusible molecules released by bacteria, and when these molecules reach high levels it triggers up regulation of gene expression that may be responsible for biofilm formation, virulence, and intake of extracellular DNA, and coping with environmental stress.[45] Ultimately, quorum sensing serves to coordinate metabolic switching within the biofilm bacteria allowing the biofilm to act as one unit versus individual bacteria. Another important characteristic of biofilms is this concept of gene transfer among bacteria. As bacteria become closely related and aggregated, there is high probability of gene uptake. Gene transfer occurs with plasmids, which are extrachromosomal DNA, that replicate independently. These linear or circular forms of DNA are not essential for bacterial survival but assist in survival under stressful conditions, such as passing along antibiotic resistance genes. This type of gene transfer occurs in bacteria in three ways: conjugation (DNA is transferred from one cell to another via cell to cell contact), transduction (during cell lyses of a bacterial cell wherein it has been infected with a virus, small bacteriophages are packaged with bacterial DNA, which can be transferred to other bacteria), and transformation (as bacteria are lysed free extracellular DNA may be incorporated into another bacterial cell). This type of horizontal gene transfer via plasmids can pass on important survival properties to other bacteria, such as antibiotic resistance, information encoded for bacteriocins, toxins, adhesins, and special metabolic enzymes.[46-53] Now that biofilm structure is defined it is important to highlight its existence in endodontic lesions. Examples from endodontic

literature divide biofilms into two areas: those that are seen extraradicular or at the apex where an endodontic lesion exists and intraradicular or within the root canal itself.

Nair et al.[54] evaluated teeth with gross carious lesions with apical inflammatory lesions after extraction with TEM. Bacterial colonies or dense aggregates were seen adhering to the root canal walls. In a similar study using SEM Molven et al.[55] demonstrated colonies of cocci organized in "corn-cob-like" structures and rods in the apical 2 mm of infected root canals. Another interesting study by Sen et al.[56] looked at extracted teeth with apical periodontitis and failed to show strong evidence of biofilms but did show root canal walls heavily infected with microorganisms (once again cocci and rods), even extending into the dentinal tubules.

Not only have efforts been made to examine biofilms inside the root canal, but extensive studies have attempted to show extra-radicular biofilms on teeth with apical periodontitis. Among these studies is one from Tronstad et al.,[43] wherein root tips associated with refractory endodontic lesions were examined under SEM. After extraction, SEM imaging revealed biofilm formation at the apex with the foramina overlaid with a smooth layer of bacteria and irregularities within the tooth structure inhabited with bacteria. All the bacteria appeared bound together by an extracellular matrix, which some authors have called extrapolymeric substance (EPS), all of which is indicative of biofilm formation.[57, 58] Lomcali et al.[59]studied primary endodontic infections and found similar outcomes as Tronstad with bacteria coating the foramina. Dense chains of multi-layered bacteria, spanning near to and at the foramina held together by EPS were examined under SEM. Once again, demonstrating biofilm formation.

Another study from Siqueira et al.[60] found 26 teeth with asymptomatic apical periodontitis that bacteria were only restricted to the root canal space and in only one tooth were bacteria found beyond the apical foramen. Bacteria colonies aggregated densely within the canal space but did not exit the canal. In another study using experimental monkeys Walton et al.,[61] showed similar findings, by examining how far bacteria infects a pulp after exposure to the oral environment. In this study, bacteria never penetrated the periapical lesion or the external apical root surface even after seven months of exposure.

However, when gutta-percha or other foreign structures, such as silver points extrude out the apex in refractory periodontitis, then biofilm formation is observed. Noiri et al.[62] observed biofilm formation in extracted teeth involved in refractory cases. Under microscopic imaging, gutta percha seen extruding from the apex was covered in EPS populated by bacteria. Similarly, Leonardo et al.[63] observed biofilm formation on extracted teeth that were involved with apical lesions. Teeth with vital pulps or necrotic pulps without apical lesions did not demonstrate biofilms. In the observed biofilms, many bacteria morphotypes were noted such as, cocci, bacilli, and filamented forms.

An important study by Nair et al.[64] highlighted the prevalence of biofilms in teeth infected with apical periodontitis. The authors treated 16 mesial roots of mandibular molars with apical periodontitis using hand/rotary instrumentation and 5.25-percent sodium hypochlorite rinses, followed by a final rinse with 17-percent EDTA, all in one step. The mesio-buccal canals were prepared with #40 Lightspeed™ NiTi rotary (Lightspeed Technology Inc, San Antonio, Tex) and the mesio-lingual canals were prepared with just hand instrumentation to a #40 hand file. Following treatment the

mesial roots were resected during apicoectomy surgery and specimens fixed, decalcified, and sectioned horizontally to be viewed under light and transmission electron microscopy. The results showed 14 of 16 teeth with persistent infection even after treatment. The microorganisms viewed under magnification were located in isthmuses, fins, lateral canals, and inaccessible recesses. And most importantly, these microbes were located in complex biofilms. From these observations, the authors concluded that microorganisms cannot be predictable eliminated by hand/rotary instrumentation alone even when larger files sizes are used. Further, they concluded a treatment plan involving meticulous instrumentation, irrigation with NaOCl, rinsing with EDTA, and the application of a microbicidal dressing for a sufficient duration of time to be effective, cannot be completed in one treatment session with contemporary technology. They found that the results of their study did not provide the biological basis for treating teeth with infected necrotic pulp in one visit.

Case reports have also highlighted the presence and role of biofilms in apical periodontitis. For example, Ferreira et al.,[65] through SEM imaging, examined the resected root of a maxillary premolar with a draining sinus tract and an apical lesion. Cocci and fungal forms were observed on the resected root tip. Carr et al.,[57] also in a case report of a mandibular first molar with refractory periodontitis and a history of long-term calcium hydroxide treatment and apical surgery, showed through transmission electron microscopy (TEM) biofilm present in each thinly sliced section. All bacteria observed was embalmed in extrapolymeric substance many layers thick, seen in isthmuses, accessory canals, dentinal tubules, and interstitial areas of dentin and fibrodentin. These diverse biofilms differed from each other in morphotype even when close to each other,

and showed an increase in ribosomal activity, suggestive of protein production and secretion. Another critical observation made in this case report, peculiar to biofilms, was bacteria blebbing, which is common in gram-negative bacteria. Blebbing, also known as membrane vesicle formation, is used to 1) establish a colonization niche, 2) transmit virulence factors, 3) modulate host defenses and responses, and 4) kill bacteria. Blebs are highly inflammatory and contain lipopolysaccharides (LPS), adhesions, toxins, and proteases and are critical in cellular interaction.

Treating biofilms is challenging, since bacteria are able to persist and grow within biofilms and endodontic success relies heavily on bacteria eradication.[5] Bacteria have been shown to resist antibiotic treatment and be protected from antimicrobial treatment when within a bioflim. Biofilms protect microorganisms, as related by Svensäter et al.[45] through the following ways:

1) Impeding antimicrobials by the structure and dense organization of the biofilm population within the polymeric matrix, leaving microorganisms in the depths of the biofilm unaffected.
2) The agent might also be inactivated in the biofilms.
3) The slow growth rate of microorganisms in biofilms can result in cells being more resistant to the agent than faster dividing cells.
4) Biofilm bacteria may also display a distinct phenotype that accounts for enhanced resistance.

Some studies have targeted antimicrobials and their effects on eliminating bacteria and as bacteria are incorporated in a biofilm, all of which has significant endodontic treatment benefits. One such study by Spratt et al.,[66] used several irrigating

solutions (2.25-percent sodium hypochlorite, 0.2-percent chlorhexidine, 10-percent providone iodine, 5 parts per million colloidal silver) to treat five common bacteria found in endodontic lesions, all within a biofilm model. Their results show sodium hypochlorite as the most effective irrigating solution in eradicating bacteria.

SUCCESS AND FAILURE

The success of any treatment requires criteria by which one can measure and qualify that success. Unfortunately, this becomes difficult and even unrealistic goal due to the many variables and factors that is required in endodontic therapy. One criterion for success is retention of a tooth without patient symptoms, a functioning tooth without symptoms, complete healing of infection, as in apical lesions, or radiographic healing of apical lesions, wherein no evidence of a lesion exists both with conventional and digital radiography as well as cone beam computed tomography. All these factors make it difficult to define success or what constitutes a failure, thus researchers have attempted to critically evaluate success. Success is critical to the endodontic therapy, because of the need for proper treatment and overall health of the patient.

Sjögren et al.[67] examined factors affecting results of endodontic treatment by evaluating 356 patients eight to 10 years after treatment. They concluded that long-term results were directly related to the preoperative periapical and pulpal status. A 96-percent success rate was noted in teeth with vital or non-vital pulps but without periapical lesions. Whereas, teeth with periapical lesions had only 86-percent success rate and teeth that required retreatment only 62 percent healed.

The Toronto study evaluated 450 endodontically treated teeth and showed a statistically significant difference in success between teeth with and without preoperative

apical periodontitis. The teeth were examined clinically and radiographically and the authors found a 92-percent success rate in teeth without preoperative apical periodontitis compared to a 64-percent rate in teeth with preoperative apical periodontitis.[68, 69]

The Washington study[25] used 1229 cases and evaluated success and failure by using radiographs at six months, one years, two years, and five years. The criteria for success were cases with "decided periradicular improvement" and "continuing periradicular health." Failure was defined as cases that "initially demonstrated periradicular damage and that had not improved, as well as those cases that had deteriorated since treatment." Statistical analysis was performed on the results accumulated at the two- and five-year intervals, since the films taken at the six month and one-year intervals were "valueless." At the two-year recall, the success rate was measured at 91.54 percent, and at the five-year recall, 93.05 percent. Further, the authors evaluated success and failure between individual teeth and found that the mandibular second molar had the highest success rate at 98 percent and the highest failure rate with the mandibular first premolar at 11.43 percent and the lateral maxillary incisors at 10.82 percent. The authors concluded the low success rates with the mandibular first premolar could be due to missed anatomy, since mandibular first premolars have been shown to have two canals 23.2 percent of the time.[70] Similarly, the maxillary lateral incisors also have been shown to have anatomic differences, such as extensive distal curvature as pointed out by Mizutani et al.[71] The most common cause for failure was incomplete obturation, accounting for 58.66 percent of the failures and root perforation at 9.61 percent, highlighting the importance of proper instrumentation and obturation. Also, cases wherein obturation occurred from 0.0 mm to 2 mm from the radiographic apex

yielded 94-percent success compared with 76 percent for those obturated beyond the apex and 68 percent for those obturated greater than 2 mm from the radiographic apex.

Salehrabi and Rotstein[72] in a retrospective study, analyzed the records from the insurance company Delta Dental of 1,462,936 teeth receiving initial endodontic therapy and assessed retention rates over an eight-year period. Overall, 97 percent of the teeth were retained over the eight-year period with 3 percent of the failures requiring apical surgery, retreatment, or extraction occurring within the first three years. Interestingly, 85 percent of the teeth extracted did not have full coronal coverage restorations, highlighting the importance of permanent restorations over endodontically treated teeth.[73] The authors concluded that initial endodontic therapy is a predictable procedure with high retention rates.

In another retrospective study carried out by Lazarski et al.[74] by assessing 110,766 in an insurance database, success rates were found to be 94 percent over a three-and-a half- year time period. Interestingly, the authors also found among a subset of 44,613 cases incidences of extraction to be 5.5 percent, retreatment of 2.77 percent, and periapical surgery to be 1.41 percent. The likelihood of extraction increased with age and with failure of the tooth to receive a coronal restoration.

Ray et al.[73] also highlighted the importance of a permanent coronal seal over an endodontically treated tooth. They randomly selected full mouth radiographs from patient's folders to evaluate teeth that had been endodontically treated by comparing the quality of both the coronal restoration and the final obturation. Of the 1010 endodontically treated teeth examined, the authors concluded that 61 percent of teeth were free of periradicular pathosis and most importantly "the technical quality of the

coronal restoration was more important than the technical quality of the endodontic treatment form apical periodontal health."

Another factor often debated as to the success of endodontic therapy is multiple versus single visit treatment. Some practitioners stress the importance of placing an intracanal medicament, such as calcium hydroxide, to allow for better cleansing of the root canal system.[75] However, others have shown there to be no statistical significant difference between multi-visit or single visit treatments as it pertains to success rates.[76] There are advantages to both treatment options with single visit requiring less time for the patient as wells as less cost and multiple visits allowing for potentially fewer flare-ups and pain.

A study by Sjögren et al.[75] investigated the outcomes of endodontic treatment on teeth with apical periodontitis and role of intracanal bacteria. Fifty-five teeth with apical periodontitis were accessed and samples taken for bacteriological sampling. The teeth were then instrumented and irrigated with sodium hypochlorite, and three more bacteriological samples were taken. Periapical healing was followed over a five-year period and healing evaluated. Interestingly, the authors found that, at the time of the first bacteriological sample, all 55 teeth were positive for bacteria. At the post-instrumentation sampling, 22 of the 55 samples yielded low levels of bacteria. After the a five-year follow up, complete healing occurred in 94 percent of the teeth that had negative cultures at the time of obturation and only 68-percent healing in teeth that were positive to bacteria at the time of obturation. These results led the authors to conclude despite the optimal endodontic therapy provided in the study, it was not possible to eradicate all infection from the root canal in one treatment visit. The authors said this

suggested that the filling of initially infected root canals should be delayed until after a "suitable period" of medication with an antimicrobial dressing.

Trope et al.[77] in a controlled prospective study evaluated the effects of multi-visit and single visit endodontic treatment on teeth with periapical lesions. One-hundred and two teeth with lesions were randomly assigned to three groups, with group one treated in one appointment, group two being treated in two visits but without any intracanal medicament being placed, and group three being treated in two visits and calcium hydroxide used as an intracanal medicament. The multi-visit groups were allowed to sit for one week and then obturated on the second visit. Evaluators using the Periapical Index Scoring System (PAI) examined the radiographs pre-treatment and post-treatment at a 52-week follow-up to compare differences between the groups. Overall, 73-percent of the teeth (75/102) finished with a good PAI score and 26 percent (27/102) had a poor PAI score. Teeth treated in two visits but without intracanal medication had poor PAI results and clearly demonstrated the ineffectiveness of this method. Seventy-four percent of teeth treated in two visits and calcium hydroxide improved as it pertains to their PAI score, whereas only 64 percent of the teeth treated in one visit improved. However, these numbers were not significant and the authors point out that for these numbers to be significant it were require a much larger sample size (for 95-percent power 571 samples). The authors found the additional disinfection with calcium hydroxide before obturation resulted in a 10-percent increase in the rates of healing.

In a recent study performed on dog teeth with apical periodontitis, Paula-Silva et al.[78] evaluated teeth endodontically treated in one visit and teeth treated in multi-visits with calcium hydroxide. They found that teeth treated in one visit had higher

inflammatory cell infiltrate, higher matrix metalloproteinase expression, and the periapical tissue was extremely disorganized. Whereas, teeth treated with calcium hydroxide demonstrated moderately organized periapical tissue, lower matrix metalloproteinase expression, lower bacteria prevalence, and less inflammatory cell infiltrate. This study showed calcium hydroxide improved the tissue repair process.

In another study comparing multi-visit treatment with calcium hydroxide compared with single-visit treatment, Weiger et al.[76] conducted a prospective clinical study treating 67 teeth diagnosed with endodontic lesions and observed over a five-year period. They found that from a microbiological perspective, one-visit root canal treatment created favorable conditions for periapical healing, and that one-visit treatment is an alternative to two-visit treatment with calcium hydroxide as an inter-appointment dressing.

Peters et al.[79] found similar results between teeth treated in a single visit and teeth treated in two visits with calcium hydroxide as an intracanal medicament for four weeks. Those treated in one visit had complete radiographic healing in 81 percent of the cases and 71 percent of the cases treated in multi-visits had complete radiographic healing. Again, as pointed out in other studies, there was no significant difference between one visit and multi-visit endodontic therapy.

ROOT CANAL ANATOMY

Root canal anatomy is complex and often different form the original perceptions one may have of a tooth. Thus, the clinician must suspect that complex anatomy, such as multiple canals, anastomeses, fins, lateral canals, and apical deltas may exist in the seemingly simple teeth. Also, understanding root canal anatomy and its complexities

plays a critical role in endodontic therapy. Incompletely debrided, disinfected, and ultimately obturated root canals can lead to endodontic failure.[80] Thus, a thorough knowledge of these canal anomalies is paramount to the treating clinician in his or her success.

Some of the earliest studies on root canal anatomy highlighted the diversity found within teeth as compared to the often over simplified perception of one canal traversing from pulp chamber to radiographic apex.[81] Some of the complexity discovered within root canal systems were C-shaped configurations, multiple canals, fins, inter-canal communications, apical deltas, and lateral canals.

Green et al.[82] demonstrated an important misconception of the terminal apical exit of the root canal. Often this apical foramen does not exit at the physical extent or radiographic apex. Green studied 400 maxillary and mandibular teeth and showed about 50 percent of the apical foramina open directly on the apex. The foramina can range anywhere within 2 mm from the radiographic apex.

Levy et al.[83] further demonstrated the eccentric location of the apical foramina by studying 122 extracted teeth. Thirty-two percent of the specimens had apical foramina with mesial and distal deviations when viewed from the buccal or lingual and 66 percent showed deviations from the maximal aspect. These findings led the authors to conclude, "due to the high frequency of occurrence of buccal and lingual deviations not seen in clinical radiographs and the magnitude of these deviations, it may be good endodontic practice to fill the root canal slightly shy of (rather than exactly flush with or past) the radiographic apex."

Another critical study in understanding the apices of teeth was performed by Kuttler.[84] Two hundred and sixty-eight teeth were collected and sectioned to evaluate microscopically the apical area, since at this time, as Kuttler states, "Up to the present time the endodontist has worked with extremely poor data." These teeth were further divided into a younger group and older group to evaluate any discrepancies. Kuttler found that with age, new layers of cementum are formed and the center of the foramen moves further away from the apical center. Also, due to the unevenness in the diameters of the foramen and the formation of a funnel shape apical to the constriction, obturating this portion would be impossible without overfilling the canal. Finally, Kuttler[84] observed discrepancies in the average thickness of the apical cementum between the younger age group and the older age group, with thicker apical cementum in the older group compared with an average thickness of 0.5 millimeters in the younger group.

Some authors have attempted to classify the root canal systems in categories for better description and clarification as it per it pertains to the many pathways a canal may traverse from the pulp chamber to the apical foramen. One of the first and highly used studies to attempt this was carried out by Weine et al.,[85] wherein the authors describe these pathways into four types:

Type I: One orifice, going to one canal and ending in one foramen.

Type II: Two orifices, going to two canals, and forming back into one foramen.

Type III: Two orifices, going to two canals, and exiting as two foramina.

Type IV: One orifice, going to two canals, and exiting as two foramina.

This simplified classification made it very easy for identification of canal systems and is in use by many still to this day.

Vertucci et al.[86] added more to the complexity of root canal classification, showing that the descriptions can be even more diverse than Weine[85] and colleagues described. Vertucci's classification included a total of eight types and these classifications are as follows:

Type I: A single canal is present from the pulp chamber to the apex.

Type II: Two separate canals leave the pulp chamber but join short of the apex to exit as one canal.

Type III: One canal leaves the pulp chamber and branches into two within the root and then merges to exit as one canal.

Type IV: Two separate canals extend from pulp chamber to apex.

Type V: One canal leaves the pulp chamber and branches short of the apex into two separate canals with separate apical foramina.

Type VI: Two separate canals exit the pulp chamber, merge in the body of the root and then branch short of the apex to exit as two canals.

Type VII: One canal exits the pulp chamber, branches and then rejoins in the body of the root, and finally branches into two distinct canals short of the apex.

Type VIII: Three separate canals extend from the pulp chamber to the apex.

While studies, as those from Weine and Vertucci have classified the diversity of the root canal system, other studies have highlighted the reality of multiple canals in teeth that often times are thought to only have one canal. For example Green[87] in his study from 1973 collected one thousand three hundred teeth and showed discrepancies in many teeth as it pertains to the number of canals. Teeth often thought to only have one canal, such as the mandibular premolar, mandibular incisors, and mesio-buccal canal of

maxillary molars, were found to have multiple canals. Other studies built upon Green's and attempted to accumulate the percentages of teeth with multiple canals, all with a wide range of percentages for all teeth in the mouth.[86, 88-94 95] Important to note from these studies is the identification of multiple canals in teeth that may appear on a radiograph to only have one canal.

INSTRUMENTATION

Instrumentation of the root canal system has been and is a major component of root canal therapy. Ingle et al.[96] mentions instrumentation as one part of what he terms the endodontic triad, which consists of canal enlargement, canal sterilization, and canal obturation. Some authors have even expressed instrumentation as the most important component of endodontic therapy.[85, 97-99] However, with all of these components being crucial, and although one would be amiss to rank one component of this triad higher than another, since they all are dependent upon each other for success, it is important to outline the evolution of instrumentation.

Schilder,[98] in discussing instrumentation, coined the term "concept of flow," which describes the shape following instrumentation as a continuous tapering funnel. He laid out five design objectives:

1) Continuous tapering funnel from the apex to access.
2) Cross-sectional diameter should be narrower at every point apically.
3) The root canal preparation should flow with the shape of the original canal.
4) The apical foramen should remain in its original position.
5) The apical opening should be kept as small as practical.

Schilder[98] continued in his description of instrumentation by highlighting five biological objectives:

1) Confinement of instrumentation to the roots themselves.
2) Do not force necrotic debris beyond the foramen.
3) Removal of all tissue from the root canal space.
4) Creation of sufficient space for intra-canal medicaments.

By fulfilling these design objectives, one could prepare a continuous taper to the root canal preparation and further enhance the goals of root canal therapy.

To accomplish proper mechanical preparation many different instruments are used. Early endodontic instruments were primitive with only excavators, iron cauterization instruments, and thin instruments being available. Edward Maynard developed the first endodontic instrument by notching a round wire, such as watch springs and piano wires which could be used for pulp extirpation.[100] But with time, endodontic instruments have evolved and can be divided into the following groups:

- Hand and finger operated instrument, such as K-files, reamers, H-type instruments, and barbed broaches.
- Low-speed instruments, such as the Gates-Glidden drills and Peaso reamers.
- Instruments that are engine driven in an electric hand piece, such as nickel-titanium rotary instruments.[99]

Not only have endodontic instruments evolved, but many instrumentation techniques have been introduced. Several hand instrumentation techniques that have been described in the endodontic literature are the step-back technique,[101] circumferential filing,[102] step-down technique,[103] and balanced force technique.[104]

Vessey et al.[105] compared the effects of a filing action (a push-pull movement) to a reaming action (a push-pull movement with a rotational component) to determine any differenced in intracanal preparations. Thirty-three extracted human teeth were prepared using the two techniques. The results of this study showed no significant deviation from a circular canal preparation between the two actions. However, a file when used in a filing action significantly deviated from a circular preparation compared to a reamer used in a reaming motion. This led Vessey to conclude that a reaming action with either a file or a reamer prepared a more circular preparation and a filing action produces more dimensional changes.

Roane and Sabula[104] introduced the balanced force technique in 1985. The technique involves instruments advancing apically with a 180-degree clockwise turn followed with 120-degree counter-clockwise turn with continued apical movement. A 120-degree clockwise turn is used on the withdrawal of the instrument. These three movements or phases are described as the placement phase, the cutting phase, and the removal phase. For a straight canal the final recommended apical size is a No. 80 or a No. 45 hand file for curved canals. The main advantages of the balanced force technique, as mentioned by Roane et al. are: good apical control, good centering of the instrument, and no need to pre-curve the instruments.

The step-back technique, as described by Clem et al.,[100] functions by preparing the apical area with small instruments and after final apical enlargement, as working length is decreased file size increases, resulting in a larger coronal flare.[101] In the step-down or crown-down technique, the orifice is first enlarged, either by files or orifice openers, and as the preparation moves apically the file sizes decrease in size, resulting in

the goals of less extruded necrotic material and less transportation.[103] However, according to Hullsman et al.,[106] there is little evidence, which supports one of these methods over the other.

Recently, with the advent of nickel-titanium and its use in hand-rotary instrumentation, there has been an increase in its use and less focus solely using hand instruments for endodontic therapy. Advantages of nickel-titanium in hand-rotary instrumentation seem to be the super-elastic property of nickel-titanium, the fast and effective canal preparation, and the maintenance of the original canal shape.[2] Hand-rotary instrumentation, as shown by Bertrand et al.,[107] out-performs just hand instrumentation in smear layer removal in the coronal and middle third of root canal systems. However, in the apical third the results between hand instruments and hand-rotary are not significantly different.

Siqueira et al.,[108] in a study evaluated whether or not hand-rotary instrumentation was more efficient in reducing bacterial loads compared to just plain hand instrumentation. Thirty-five extracted human teeth, after being incubated with *E. Faecalis*, were prepared with either different engine-driven, hand-rotary instrumentation or just hand instrumentation without the aid of intracanal medicaments. Root canals were sampled before and after instrumentation and showed that all groups removed more than 90 percent of bacterial cells, regardless of instrument. The authors concluded that while larger preparations could incorporate more anatomical irregularities and allow the removal of more bacterial cells from the root canal, the mechanical means are insufficient to completely eradicate root canal infection. Therefore, they said, the use of adjunct chemical substances with antibacterial properties is necessary.

In a similar study, Dalton et al.,[2] also attempted to compare nickel-titanium, hand-rotary instrumentation to conventional hand instrumentation using stainless-steel hand files in a step-back technique with regards to intracanal bacterial reduction. Patients with apical periodontitis received endodontic therapy either by the use of nickel-titanium, hand rotary instruments (Profile .04) or stainless-steel K-files with sterile saline as an irrigating solution. Samples, taken from the patients before and after instrumentation, were evaluated to see the bacterial reduction load. As in the Siqueira study, Dalton et al. found no statistically significant difference between the two groups and that as larger file sizes are used, fewer bacteria remain in the root canal system.

Ultimately, the endodontic literature shows, mechanical preparation of the root canal results in a cleaner canal wall in the coronal one-third, as well as some bacterial reduction. But difficulty still remains in thorough cleansing of the apical one-third of the canal, as well as complete bacterial reduction through mechanical preparation alone. Ingle et al. highlighted this important point in a study, wherein cultures were taken before and after mechanical preparation with sterile saline. They concluded that, "mechanical instrumentation does not render the root canal "sterile," although it may reduce temporarily the number of microorganisms… [i]t remains for antibacterial medication to destroy the bacterial." [96] Clearly, this study establishes the need for not just mechanical preparation, but chemo-mechanical preparation.

IRRIGATING SOLUTIONS

Seltzer et al.[109] in an article evaluating the effects of different intracanal medicaments outlined the major role and functions of medicaments used in endodontic therapy. An ideal intra-canal medicament should:

1) Eliminate or reduce microbial flora.
2) Prevent or lessen pain.
3) Reduce inflammation.
4) Stimulate repair.

Although this article precedes many of the articles wherein endodontic irrigating solutions were substantiated, these roles still are current today.

In another evaluation of intra-canal medicaments, Torneck et al.[110] also highlighted the roles of an ideal irrigating solution, but extended the criteria to include:

1) It must be rapidly effective in eliminating or destroying those microorganisms normal found in the root canal.
2) It must be effective in destroying, neutralizing, or culminating any toxic products of which may be present in these canals.
3) It must be non-staining to tooth tissue.
4) It must have good penetrating quality in order to be effective deep within the dentinal tubules.
5) It must remain stable at room temperature for long periods of time.
6) It must not be inactivated by blood, serum, protein, pus, or other organic matter of which may be present within the root canal or periapical tissues.
7) It must be readily available.
8) It must be easy to use.

With these roles in mind many different irrigating solutions have been employed in chemo-mechanical preparation. Sodium hypochlorite has become the irrigating solution of choice for many practitioners.

Sodium hypochlorite is has been used in endodontics for many years as an intracanal irrigating solution, because of its physical characteristics that allow it to remove necrotic and vital tissue, eliminate bacteria, and remove dentinal debris that accumulates during endodontic instrumentation.[111] Sodium hypochlorite has been shown to have a pH between 10.0 and 11.0 and as it comes in contact with water it dissociates into sodium and hypochlorite ions, which can combine with hydrogen to form hypochlorous acid. This acid has been thought to be bactericidal and disrupt oxidative phosphorylation on cellular membranes and DNA synthesis.[112-114] Sodium hypochlorite comes in concentrations ranging from 0.5 percent to 6.0 percent, with tissue dissolution being better as the concentration increases.[115] Because of its universal use in endodontics, sodium hypochlorite has been extensively study in the literature.

In a study conducted by Waltimo et al.,[116] differing concentrations of sodium hypochlorite were studied against *Candida albicans*. Although a bench top study, the authors found that 5.0-percent and 0.5-percent sodium hypochlorite solutions killed the all the yeast cells in 30 seconds or less. However, lower concentrations of sodium hypochlorite were not as effective. Similarly, Vianna et al.[117] tested sodium hypochlorite against different bacteria, such as *P. gingivalis, P. intermedia, S. Aureus,* and *E. faecalis,* and found that all the concentrations of sodium hypochlorite (0.5 percent to 5.25 percent) were effective at eliminating these organisms within 30 seconds. The only difference, being that as the concentration increases the amount of time to eliminate the microorganisms decreased.

Gomes et al.[118] also tested different concentrations of sodium hypochlorite against *E. faecalis* to evaluate the amount of time needed to completely eradicate the

microorganism from the culture. The results from this study confirm the previous studies, which is sodium hypochlorite (0.5 percent to 5.25 percent) can kill bacteria. Interestingly, 0.5-percent sodium hypochlorite required almost 30 minutes contact time to eliminate *E. faecalis*, whereas it required less than 30 seconds for a concentration of 5.25 percent. Radcliffe et al.[3] found in a similar study designed evaluated sodium hypochlorite against a small range of bacteria. They also found that with increased concentrations of sodium hypochlorite there was faster elimination of the microorganisms. However, in contrast to Gomes et al.,[118] they also found a higher resistance of *E. faecalis* to sodium hypochlorite, and they hypothesized that, because of this resistance, it "may result in its surviving dental root canal treatment and subsequently being associated with refractory infection."

One benefit of sodium hypochlorite has been its efficacy in dissolving organic tissue. Hand et al.[115] evaluated differing concentrations of sodium hypochlorite and its tissue dissolving effects on necrotic tissue. Using Sprague-Dawley rats, tissue was collected and frozen for further testing. This necrotic tissue was exposed to 5.25, 2.5 1.0 and 0.5-percent sodium hypochlorite, normal saline, distilled water, and 3.0-percent hydrogen peroxide. The results demonstrate that dilution of sodium hypochlorite results in decreased efficacy on necrotic tissue. Five-point-two-five percent sodium hypochlorite is significantly more effective than 2.5-, 1.0-, and 0.5-percent sodium hypochlorite, distilled water, normal saline, and 3.0-percent hydrogen peroxide. Two-point-five percent sodium hypochlorite showed better results as a necrotic tissue solvent than 1.0-percent and 0.5-percent sodium hypochlorite. The author's final conclusion of the results state, "available scientific evidence suggests that dilution of 5.25-percent

NaOCl adversely affects its necrotic tissue dissolution property, its antimicrobial property, and its ability to aid in the debridement of the canal system."

To try and simulate clinical conditions in testing irrigation solutions, Siqueira et al.[119] used human extracted teeth infected with *E. faecalis*. These teeth were then accessed and inoculated with a broth of *E. faecalis*, and divided into groups to be instrumented and irrigated with 1, 2.5 and 5.25 percent sodium hypochlorite or saline solution. Bacterial culturing samples were taken prior to instrumentation and irrigation to compare the groups. The results of this study show no statistically significant difference between the different sodium hypochlorite groups, but statistical difference did exist between the sodium hypochlorite groups and the saline solution as it pertained to eliminating *E. faecalis*. This study highlights the importance of an antimicrobial during endodontic therapy but it also points out bacteria in a root canal system, although significantly reduced, is not completely eliminated by chemo-mechanical preparation.

Byström et al.[120] conducted one of the first *in-vivo* studies that investigated the effectiveness of sodium hypochlorite on bacterial elimination. Patients with necrotic pulps were treated with chemo-mechanical, non-surgical root canal therapy and use of either 0.5-percent sodium hypochlorite or physiological saline. The results from this study demonstrate that in twelve of the fifteen teeth treated with sodium hypochlorite, no bacteria could be recovered. However, in the teeth treated with saline, eight of 15 root canals showed no bacteria cultures. The authors concluded that 0.5-percent sodium hypochlorite is more effective than saline as a root canal irrigant. Although the results from their study did not challenge the results of other research into the effectiveness of

sodium hypochlorite in eliminating bacteria, Byström et al. did show the properties of sodium hypochlorite's use *in-vivo*.

In another *in-vivo* study, Peciuliene et al.[121] evaluated chemo-mechanical preparation of previously treated teeth with apical periodontitis, using sodium hypochlorite as an irrigating solution. Microbiological sampling was taken prior to treatment and following chemo-mechanical preparation. The results from this study show that bacteria was found in thirty-three of the forty teeth prior to instrumentation. After instrumentation, no gram-negative rods or yeasts were present in microbiological culturing, microbes were found in five other teeth, and *E. faecalis* was present in six out of the twenty-one teeth originally cultured. This again highlights the effectiveness of chemo-mechanical preparation with sodium hypochlorite. Also important is the persistence of *E. faecalis* in refractory apical periodontitis and the possible resistance to sodium hypochlorite.

Other authors also have highlighted resistance demonstrated by some bacteria to the effects of sodium hypochlorite. Haapasalo et al.[122] revealed that dentin powder in the presence of 1.0-percent sodium hypochlorite showed delays in the killing of *E. faecalis*, suggestive that *in-vivo* sodium hypochlorite may not be as effective. Another disadvantage of sodium hypochlorite is its lack of property to completely remove the smear layer. [123]

Baumgartner et al.[124]evaluated under SEM teeth chemo-mechanically prepared to better understand smear layer. The authors concluded the following about smear layer:

1) The smear layer is two components: a thin layer of smear material on the surface of the canal wall and smeared material packed into dentinal tubules.

2) The smear layer is usually one to two micrometers in thickness.
3) The smeared material in the dentinal tubules occasionally was packed up to forty micrometers.
4) The frequency of smeared material packing in dentinal tubules is unpredictable.
5) The smeared material, which results from instrumentation, appears to be friable and loosely adherent to the dentinal tubules.

Biofilms in endodontic infections has created a paradigm shift in the role of irrigating solutions. Biofilm models have been researched in the solutions effectiveness to eliminate bacteria within a biofilm. Chavez de Paz et al.[125] evaluated the effects different antimicrobials had on bacterial elimination when associated in a biofilm. The authors created *in-vitro* biofilms inhabited by bacteria. These biofilm models containing bacteria were exposed for five minutes to the following antimicrobials: alkali (pH of 12), 2.5- percent chlorhexidine, fifty mmol/L EDTA, and 1.0-percent sodium hypochlorite. The results demonstrated 1.0-percent sodium hypochlorite affected the membrane integrity of all the microorganisms and removed most the biofilm cells. EDTA was unable to remove more than a few cells in the biofilms of the bacteria, but it did affect the membrane integrity in all the microorganisms. Chlorhexidine had a minimal effect on membrane integrity of *E. faecalis* and only eliminated 50 percent of its biofilm cells. Alkali was the least effective antimicrobial on the biofilms. In conclusion the authors stated "[o]ur findings show that biofilm structure and susceptibility to antimicrobials is affected by a number of factors such as the surrounding nutrient environment and the substratum."

Clegg et al.[126] also studied the antimicrobial effects of irrigating solutions on bacteria associated with biofilms. In this *in-vitro* study, intracanal contents from patients with chronic apical periodontitis was collected and placed on sectioned, extracted human teeth. With placement of the intracanal contents, a polymicrobial biofilm formed on the surfaces of the sectioned area. These teeth were then immersed for fifteen minutes in the following antimicrobial agents: 6.0-percent sodium hypochlorite, 3.0-percent sodium hypochlorite, 1.0-percent sodium hypochlorite, 2.0-percent chlorhexidine, and 1.0-percent sodium hypochlorite followed by Biopure MTAD™. SEM imaging revealed that 6.0-percent NaOCl and 3.0-percent NaOCl disrupted and removed the biofilm. One-percent NaOCl, 2.0-percent chlorhexidine, and 1.0-percent NaOCl followed by MTAD disrupted the biofilm but did not eliminate bacteria. Two percent chlorhexidine was not able to disrupt the biofilm or eliminate bacteria. Also, viable bacteria was not cultured after being exposed to 6.0-percent NaOCl, 2.0-percent chlorhexidine, or 1.0-percent percent NaOCl followed by MTAD. Clegg et al. found that 6.0-percent sodium hypochlorite was the only agent capable of both physically removing artificial biofilm and killing bacteria.

Sodium hypochlorite also is toxic to the cells and human tissue. Symptoms that follow a sodium hypochlorite accident are: pain, edema, hematoma, ecchymosis, hemorrhage, swelling, abscess, paresthesia, and anesthesia. Pashley et al.[127] studied the potential risks and harmful effects of sodium hypochlorite on human blood samples, rabbit eye tissue, and rat skin. The results of their study highlight the cytotoxic effects of sodium hypochlorite, which was complete hemolysis of red blood cells, severe irritation to the rabbit eyes, and skin ulcerations in the rats. The authors concluded that although

sodium hypochlorite dissolves proteins efficiency, it "is extremely cytotoxic and should be used judiciously and with caution in endodontic therapy."

Due to the potential toxic and caustic nature of demonstrated with the use of sodium hypochlorite, much has been studied and written about chlorhexidine (CHX) gluconate as an alternative antimicrobial. With a broad antimicrobial spectrum, CHX has been shown to be effective against Gram-positive and Gram-negative bacteria and yeasts. However, viruses, mycobacteria, and spores seem to be resistant to CHX. This irrigating solution penetrates the cell walls of bacteria and yeast, invading and destroying the inner cytoplasmic membrane, thus killing them. Because of these antimicrobial effects, CHX has become an important irrigating solution in the endodontic literature.[112, 128-130]

One study by Gomes et al.[118] showed the promising results chlorhexidine has as it pertains to antimicrobial activity. This bench top study evaluated different concentrations of chlorhexidine and sodium hypochlorite and the contact time required to eliminate *E. faecalis*. Although all the concentrations of both types of irrigating solutions eventually killed *E. faecalis*, the duration of time was different ranging from 30 seconds to two hours depending on the concentration. The fact that chlorhexidine, especially 2.0-percent chlorhexidine eliminated *E. faecalis*, highlights its role as an alternative endodontic irrigant. However, important to note from this study, is that there was no significant difference between the highest concentration of chlorhexidine (2.0 percent) and sodium hypochlorite (5.25 percent). Both eliminated *E. faecalis* in less than 30 seconds.

Oncag et al.[131]conducted an *in-vitro* and *in-vivo* study again evaluating the effects of both sodium hypochlorite and chlorhexidine. In the *in-vitro* study extracted human incisors were mechanically prepared and *E. faecalis* used to contaminate them. Differing

concentrations of sodium hypochlorite and chlorhexidine were used as an irrigating solution. The results showed 2.0-percent chlorhexidine was significantly more effective on elimination of *E. faecalis* at five minutes then 5.25-percent sodium hypochlorite. In the second part of this study, ninety-one root canals from necrotic deciduous teeth were endodontically treated on an initial visit. A bacterial sample was taken upon access into the canal. Irrigating solutions (same as those used in the *in-vitro* portion) were delivered during this first visit, following which the canals were temporarily sealed with glass ionomer and no intracanal medicament. Forty-eight hours later the patient returned to obturate the teeth. At this visit another bacterial sample was taken to evaluate the residual effects of the irrigating solutions from the first visit. The results from this part of the study showed 2.0-percent chlorhexidine significantly more effective in reducing anaerobic bacteria then 5.25-percent sodium hypochlorite. And in a final phase of this study, the authors evaluated the cytotoxicity of both chlorhexidine and sodium hypochlorite after being injected subcutaneously into rats. After two weeks, sodium hypochlorite showed greater toxicity when compared to chlorhexidine. The authors concluded from these results that 2.0-percent chlorhexidine gluconate displayed residual antibacterial activity and was more powerful and less toxic than 5.25-percent sodium hypochlorite; they found that 2.0-percent chlorhexidine gluconate was preferred as an irrigation solution during root canal treatment of deciduous teeth.

Due to the complex environment in which endodontic infections occur, irrigating solutions can loose efficacy and fail to eliminate microbes. Portenier et al.,[132, 133] in two studies, evaluated inorganic and organic substances and their effects on chlorhexidine. These studies revealed that inorganic substances such as dentin chips slowed down it

efficacy in killing *E. faecalis*. Also, serum albumin and heat killed microbial cells down regulated chlorhexdine in its potential to eliminate *E. faecalis*. These results suggest that with the many dentin, bacteria, and proteins involved in an endodontic infection, there is a possibility that chlorhexidine will lose its antimicrobial properties.

Although Oncag et al.[116] concluded the benefits of chlorhexidine over sodium hypochlorite, other studies have shown that they are equally effective in eliminating *E. faecalis*[134-136] as well as with killing *C. albicans*. From these studies it is apparent that, although chlorhexidine may be used as an alternate or adjunct irrigating solution, it is not clinically more effective than sodium hypochlorite.

Ethylenediamenetetraacetic acid (EDTA) is another irrigating solution that has received considerable attention in the endodontic literature. Following mechanical preparation of the root canal wall, a layer of debris, often termed a smear layer, forms. Boyde et al.[137] first called this layer of debris the smear layer and McComb et al.[138] noted this layer after the instrumentation of root canals. The smear layer, under SEM, appears amorphous and irregular, consisting of dentin debris, remnants of pulp of tissue, pulp tissue and bacteria. This layer thus consists of both inorganic and organic material. EDTA is a chelating agent that functions by demineralizing hard tissue such as dentin.

Nygaard Östby[139] first introduced EDTA to the dental community in 1957. In this article Östby described the use of EDTA during cases wherein endodontic therapy was needed. The benefits of EDTA, as explained by the author, were it seemed to reduce the time of debridement and reaming significantly, narrows canals were more easily negotiated with its use, and fractured instruments were more easily by-passed with it. Also, the author histologically studied the periapical tissues of teeth for which EDTA was

used and found no adverse reaction during routine use and no deleterious effect on pulpal or periapical tissues.

Stewart et al.[140] studied both *in-vivo* and *in-vitro* the effects of EDTA. Dye penetration studies were performed on teeth that had been rinsed with EDTA. More dye penetrated the dentinal tubules when EDTA had been used prior to the dye placement. From this observation, the authors felt that with the use of EDTA during endodontic therapy this might open the dentinal tubules and allow better penetration of intracanal medicaments. In the *in-vivo* portion of the study, EDTA was used in 140 teeth during endodontic therapy. When EDTA was used in combination with urea peroxide, it was effective in cleaning and preparing the root canal. Also this combination was a successful chelating agent and debris was often times observed floating during treatment.

In anther study evaluating the effects of different concentrations of EDTA, Patterson[141] carried out a multi-phase study involving human extracted teeth, rats, microbiological study, and an *in-vivo* study. The human extracted were exposed to varying concentrations of EDTA ranging from .03 percent to 15 percent. After exposure to the EDTA, tooth hardness was determined and quantified. The teeth exposed to EDTA exhibited surface etching with the overall hardness of the teeth decreasing. EDTA also demonstrated substantivity effects when left in the canal and sealed, causing decalcification which was not self-limiting. Also the author injected rats with different concentrations of EDTA and found that 15-percent EDTA exhibited extreme inflammation. Microbiological samples of bacteria exposed to EDTA revealed a zone of inhibition of bacterial growth. In the final phase of the study, two hundred patients were

treated endodontically with the use of EDTA. None of these patients reported any post-operative discomfort with its use.

Byström et al.[142] studied the use of EDTA in conjunction with sodium hypochlorite. Sixty teeth with necrosis and periapical pathosis were treated in this study. These teeth were chemo-mechanically treated and then received one of three irrigating protocols (group one: 0.5-percent sodium hypochlorite; group two: 5.0-percent sodium hypochlorite, and group three: 0.5-percent sodium hypochlorite together with 15-percent EDTA). Cultures were taken and from this the effectiveness of these irrigating protocols was examined. The results indicated that there was no difference between the two sodium hypochlorite solutions when used independently, but the 0.5-percent sodium hypochlorite, 15-percent EDTA combination was more efficient in eliminating bacteria.

Yamada et al.[9] studied the chelation effects of EDTA by using forty human extracted teeth and chemo-mechanically preparing the root canals with constant flushing of 5.25 percent of sodium hypochlorite. The teeth were divided into a control group and seven experimental groups to be irrigated with the following solutions: physiologic saline, 5.0-percent sodium hypochlorite, 17-percent and 8.5-percent EDTA, and 25-percent citric acid. After the final irrigation protocol, the teeth were split longitudinally, coated with gold, and imaged using SEM. The results show that saline solution does not effectively clean the canal. Five-percent sodium hypochlorite alone can clean the canal but cannot remove the smear layer. The chelating agents alone could remove the smear layer but could not completely clean the canal, leaving varying amounts of debris. Sodium hypochlorite with 25-percent citric acid was not as consistent in debris smear layer removal as the EDTA and sodium hypochlorite, but they were effective at smear

layer removal. There was also the presence of bacteria and crystal formations in this group. The combined use of 17-percent EDTA and 5.25-percent sodium hypochlorite, revealed the best results in cleaning the canal and removing the smear layer.

ULTRASONICS

As is evident from all the research done on irrigating solutions, they are paramount in the chemo-mechanical phase of endodontic therapy. However, it is critical for these solutions to reach all parts of the root canal. Many teeth exhibit difficult anatomy, such as curvature, dilacerations, fins, isthmuses, and lateral canals. And without the solutions contacting these areas physically, then their efficacy is obsolete. Chow et al.[143] showed little fluid exchange occurred and debris removal occurred beyond the tip of the needle. McGurkin-Smith et al.[144] showed that the final apical preparation size was critical in eliminating bacteria in the apical third. Larger apical preparations revealed more bacteria removal than smaller diameter preparations. Nguy et al.,[145] in studying irrigation exchange in curved canals, showed irrigation was significantly less effective. Senia et al.[146] highlighted the benefits of sodium hypochlorite in tissue dissolution but failed to show tissue dissolution in the apical 5 mm. Canals in mesial roots of mandibular molars were not adequately cleaned by sodium hypochlorite and more debris was noted in the isthmus.

Because of the inability of irrigating solutions to reach these difficult to reach areas, many studies have been undertaken to find alternative ways of delivery irrigating solutions. Ultrasonic and sonic devices have been employed in hopes of bettering irrigating delivery efficacy. Ultrasonic devices function by vibrating at or faster than the speed of sound (oscillation frequency of 18,000 to 30,000 cycles per second), whereas

sonic devices oscillate at a frequency of 1500 to 6500 cycles per second. Files, stainless steel tips, or plastic tips can be attached to these devices and placed in the canal to assist in the delivery of irrigating solutions. The debridement caused by ultrasonics was thought to occur by two actions, cavitation and acoustic streaming. Cavitation is the growth and collapse of small gas-filled bubbles, which results in energy from the collapse that may disrupt debris from the canal walls. Acoustic streaming is the rapid movement of particles of fluid in vortex-like motion around a vibrating file.[147, 148] However, ultrasonic cavitation was later proven to not play a role in canal cleaning and acoustic streaming was shown to be the main mechanism involved.[17] In the literature, ultrasonic irrigation can be further divided into two groups: ultrasonic irrigation (UI) and passive ultrasonic irrigation (PUI). The distinction between the two hinges on whether the actual ultrasonic file or tip is contacting the wall, as in UI, and mechanically preparing the canal wall. PUI functions by the ultrasonic tip oscillating within the canal without contact and without mechanically preparing the canal through ultrasonic oscillation. The main function of PUI thus being a oscillating file or tip vibrating at ultrasonic frequencies, creating a vortex solutions to debride the canal wall.[147] Most literature with the use of ultrasonic activation being published in the literature reviews the action of PUI.

Sjögren et al.[149] in a clinical evaluated the antimicrobial effects sodium hypochlorite can have when used in conjuction with ultrasonic activation. Teeth with periapical lesions were chemo-mechanically prepared and microbiological samples taken to evaluate the effects of ultrasonically activating 0.5-percent sodium hypochlorite versus hand instrumentation alone. The results demonstrated that ultrasonic activation improved root canal disinfection over hand instrumentation. In prospective, randomized, single-

blinded clinical study, Carver et al.[13] chemo-mechanically prepared the thirty-one mesial roots of necrotic mandibular molars. These mesial roots were chemo-mechanically prepared and randomly received either hand instrumentation with sodium hypochlorite irrigation or an additional one-minute ultrasonically activated irrigation with sodium hypochlorite. Culturing methods were employed during the treatment and demonstrated a reduction in CFU count in the teeth treated with an additional one-minute ultrasonic activation. The authors concluded that a one-minute ultrasonic activation was seven times more likely to yield a negative culture. However, in a bacteriological comparison of ultrasonic and hand instrumentation of root canals in dogs, DeNunzio[150] failed to show significant difference between the two treatment options. Dogteeth were inoculated with bacteria then received either hand instrumentation alone with sterile saline flushes or ultrasonically prepared with sterile saline. The authors summarized that these two treatment options were equally effective in removing bacteria from the root canal.

Other ultrasonic studies, instead of showing microbiological reduction load, have attempted to show the effects ultrasonic activation has on debris removal in teeth. Cunningham et al.,[151] using eleven pairs of extracted human teeth, instrumented one of each pair with K-files and 2.5-percent sodium hypochlorite. The other half-of-teeth were instrumented with size 10 and size 15 files using ultrasonics and 2.5-percent sodium hypochlorite. Afterwards the teeth were demineralized and evaluated under light microscopy at different levels 1 mm to 3 mm from the apex. At the 3-mm and 5-mm levels, ultrasonically instrumented teeth were the cleanest in all cases. At one millimeter from the apex, 10 of 11 teeth instrumented with ultrasonics showed better debridement.

The authors found that the root canals of the teeth ultrasonically filed and irrigated were found to be significantly cleaner.

Goodman et al.[152, 153] also histologically examined mesial roots of extracted mandibular molars after either receiving step-back instrumentation or step-back instrumentation and ultrasonic activation with 2.5-percent sodium hypochlorite. Teeth were sectioned at 1 mm and 3 mm from the apex to compare isthmus cleanliness between the two canals in the mesial roots. The result of this study showed that the step-back/ultrasonic technique was significantly better in cleaning the isthmus than the step-back technique alone. Cameron et al.[152] also studied the synergistic relationship sodium hypochlorite and ultrasonic activation could have on canal debridement. Single rooted human extracted teeth were chemo-mechanically prepared and then divided into five groups, receiving either just 4.0-percent sodium hypochlorite flush for three minutes alone or ultrasonic activation with 4.0%, 2.0%, 1.0%, or 0.5% sodium hypochlorite for three minutes. The teeth were longitudinally sectioned and studied under SEM. The results showed smear layer still present on the teeth that received just a flush with 4.0-percent sodium hypochlorite. Ultrasonic activation with 4.0-percent and 2.0-percent sodium hypochlorite showed better smear layer removal, whereas 1.0-percent and 0.5-percent ultrasonic irrigation still showed smear layer present on all surfaces. Thus, "a synergistic relationship does exist between ultrasound and sodium hypochlorite, and this relationship is clinically significant when solutions containing 2.0% available chlorine are used."

Another study by Walker et al.[154] compared the difference between just regular tap water and sodium hypochlorite when used in conjunction with ultrasonic activation.

Twenty extracted human mandibular molars with canal curvature between eighteen and thirty-five degrees were either prepared using ultrasonics and regular tap water or ultrasonics and sodium hypochlorite. The teeth were then stained and evaluated histologically, showing that sodium hypochlorite was more effective than tap water in removing tissue debris in root canals.

Guerisoli et al.[155] evaluated smear layer removal by EDTA and sodium hypochlorite with ultrasonic activation. Twenty extracted mandibular incisors were mechanically prepared then treated with ultrasonic activation using different irrigating solutions. The teeth were sectioned and examined under SEM. The results showed that with ultrasonic activation, sodium hypochlorite with EDTA removed more smear layer from root canal walls. And irrigation with distilled water or sodium hypochlorite alone did not remove the smear layer.

A clinical study by Archer et al.[12] evaluated the *in-vivo* debridement efficacy of a step-back technique compared to a step-back and ultrasound technique. Mesial roots of mandibular molars were prepared using these two techniques, then extracted and evaluated by histological means. The isthmuses were significantly cleaner at the one and three millimeter levels using the step-back and ultrasound technique. In a similar *in-vivo* prospective, randomized, single-blinded study carried out by Burleson et al.,[11] human necrotic mandibular molars were chemo-mechanically prepared. The mesial roots were divided into one of two groups, either receiving just hand and rotary instrumentation with a fifteen milliliter flush of 6.0-percent sodium hypochlorite or both hand and rotary instrumentation with a one minute ultrasonic activation with 6.0-percent sodium hypochlorite. The ultrasonic device used in this study projected a steady flush of 15 ml

of sodium hypochlorite over the one minute. Teeth were then extracted and cut in 5-um sections at 11 different levels from 1 mm to 3 mm from the apex to be examined under light microscopy. The isthmuses between the two canals were significantly cleaner at all eleven levels in the teeth treated with hand/rotary instrumentation followed by ultrasonic activation compared to just hand/rotary instrumentation. The authors concluded that a "one-minute use of ultrasonically activated irrigation, following hand/rotary root canal cleaning and shaping, has been shown to improve canal and isthmus cleanliness in terms of necrotic debris/biofilm removal."

Recently much has been written about sonic activation, due to the emergence of the EndoActivator™ (Dentsply Tulsa Dental Specialties, Tulsa, OK). This sonic device oscillates at a lower frequency (1-6 Hz) and utilizes a polymer disposable tip that comes in three sizes. Tronstad et al.,[156] in one of the first studies looking at sonic activation within a root canal compared the effects of it to chemo-mechanical hand instrumentation. Forty-nine roots of dogs were treated *in-vivo* with these two treatments and then extracted, split longitudinally, and examined under SEM. The authors found that sonic activation with EDTA removed the smear layer but with 2.5-percent sodium hypochlorite it did not. And compared to chemo-mechanical hand instrumentation, sonic activation appeared to be as similar when the root canal walls were viewed under SEM.

Many studies not only have attempted to compare hand instrumentation to sonic activation but also have compared PUI to sonic activation. One such study by Stamos et al.[157] used the mesial roots of extracted human mandibular molars. These roots were divided into the following groups: hand instrumentation, sonic activation with water, PUI with water, and PUI with 2.6-percent sodium hypochlorite. After this treatment, the teeth

were horizontally cut into sections from the apex to the coronal third of the canal to evaluate under a light microscope the amount of debris removed between the two canals in the mesial root. The results showed that at the three millimeter level there was not significant difference in canal and isthmus cleanliness between the groups. However, at the one-millimeter level, PUI with 2.6-percent sodium hypochlorite showed significantly greater percentage of canal debridement than the other groups. PUI was significantly better than sonic activation at cleaning the canal. And sonic activation was not significantly better at cleaning the canal than hand instrumentation, however, sonic activation and PUI were significantly faster in canal preparation.

In a similar study, Jensen et al.[158] also used extracted human molars and treated these teeth with hand instrumentation, hand instrumentation plus PUI, or hand instrumentation plus passive sonic activation. But instead of evaluating the teeth in cross sections, these teeth were split longitudinally and divided in a grid pattern to be evaluated under SEM for debris remaining. The scores were analyzed and showed that debris scores for both sonic and ultrasonic activation were significantly lower than the hand instrumentation group. There was not any significant difference between PUI and passive sonic activation. Ultimately, the authors concluded that passive sonic activation after hand instrumentation was more effective at cleaning the canal walls than hand instrumentation alone and was comparable to PUI.

In a recent study, Al-Jadaa et al.[159] used a model where simulated curved canals were constructed out of epoxy models and filled with necrotic bovine pulp tissue. Three passive ultrasonic set ups (straight stainless steel files, pre-bent stainless steel files, and nickel-titanium files) and a passive sonic device with plastic tips were used in attempts to

remove the pulp tissue. The authors concluded that under this type of model, PUI was significantly more effective than sonic activation in tissue dissolution. Sabins et al.[160] also found PUI to be more effective than sonic activation in debris removal from a canal wall.

Another recent study by Jiang et al.[161] developed a model to compare debris removal between PUI and passive sonic activation. Extracted human canines were embedded in acrylic, sectioned longitudinally, sanded flat on the walls containing the root canals, and then reassembled back together using the acrylic model. These reassembled teeth were then mechanically prepared with hand and rotary files. The grooves created were then filled with dentin debris to be either sonically activated or ultrasonically activated with irrigating solutions. The results showed that 89 percent of the canals with dentin debris were completely free of debris when PUI was used, whereas only 5.5 percent to 6.7 percent of the canals were free of debris with the use of passive sonic activation. These results were significant and led the authors to conclude, "that activation of the irrigant enhances the removal of dentin debris from the apical root canal."

From the literature it can be summarized that PUI achieves the following points:

1) PUI was more effective than syringe needle irrigation in removing pulpal tissue remnants and dentin debris.[11, 152, 153, 160, 162, 163]
2) PUI was capable of removing more debris than sonic irrigation.[158, 163, 164]
3) PUI was more effective in removing smear layers with sodium hypochlorite than when PUI was used with water as an irrigating solution.[152, 165-167]

4) PUI when used with sodium hypochlorite and EDTA as irrigating solutions was effective at removing smear layer, especially in the middle and cervical thirds of the root canal.[152, 155, 166, 168]
5) PUI when used with sodium hypochlorite and EDTA as irrigating solutions was not as effective at removing smear layer in the apical third of the root canal.[169-172]
6) PUI after hand or rotary instrumentation resulted in a significant reduction of the number of bacteria when compared to just hand or rotary instrumentation with syringe needle irrigation.[13, 167, 173, 174]

These points highlight the importance of delivering the irrigating solutions to all areas of the root canal system. However, there are still limitations with PUI and thus other delivery systems are available in the market to overcome some of these limitations.

EVALUATION OF POST-OPERATIVE ROOT CANAL CLEANLINESS

Most studies that evaluate the effectiveness of canal cleansing, whether through mechanical or chemical means, have utilized either histological evaluation or SEM examination. The former requires horizontal sections of the tooth that are stained and then viewed under light microscope to evaluate the canal walls for debris removal. Studies, like these, give great insight and view of the canal isthmuses, fins, and anastomeses, but fail to quantify the amount of debris removal.[11, 54]Some drawbacks of histological sectioning is the inadvertent loss or removal of debris that may occur during the sectioning and staining. Other studies have incorporated the use of SEM to evaluate and quantify the amount of debris removal from a canal wall. Various methods of

quantification have been employed, such as viewing the image under a grid system,[175]counting the amount of open dentin tubules, scoring a section subjectively based on the amount of debris present or absent,[176 177]or assigning a score, such as a three-, four-, or seven-point scoring system based on the amount of smear remaining. Critics of SEM point out the high possibility of debris being introduced into the root canal systems during the longitudinal sectioning, as well as the potential damage to the canal walls.[106, 178-180]

Controversy exists with the removal of the smear layer and its overall affect in endodontic therapy underscored by some critics of studies which focus on smear layer removal.[181-184] These authors highlight the unknown clinical significance of the residual debris and smear layer and regard the presence of residual debris as a poor outcome measure. Although a marker of canal cleanliness, the amount of residual debris cannot be specified "pre-operatively" and therefore has no clinical relevance, the authors said.[185] Khot et al.[186] discussed that for root canal isolates to grow it is essential that they be exposed to blood, serum, and saliva and these isolates did not live on pulp tissue and dentin alone. However, in defense of smear layer removal, it is important to point out that evidence has supported its removal due to undesirable effects, such as smear layers may harbor microorganisms,[138, 181, 187] the prevention of medicaments into dentinal tubules,[187, 188] and the inability to properly seal during obturation.[189, 190]

MATERIALS AND METHODS

Eighty human, single-rooted, anterior maxillary and mandibular were used during this study. These teeth were collected from the Oral Health Department under IUPUI/Clarian IRB study number 0306-64 and from local oral surgeons within the area of Indianapolis, IN. Radiographs were taken of all teeth to assure they had single canals, no abnormal root canal anatomy such as, curvature less than thirty degrees as evaluated by Schneider's method and pulp calcifications. Teeth were sterilized in 6.0-percent sodium hypochlorite for a two-week period. Following the selection and evaluation of the teeth, they then were randomly divided into four groups of 20 teeth.

ROOT CANAL PREPARATION

An access opening was performed using a new #556 carbide bur (SS White Burs, Inc. Lakewood NJ) (Figure 1) and working length determined with a new #10 stainless steel K-file (Dentsply Maillefer, Tulsa OK) for each tooth (Figure 2). The incisal edge was the reference point and the #10 file was instrumented through the apex and then brought back 1-mm to be the final working length (Figure 3). Rotary instrumentation was performed using the EndoSequence nickel titanium rotary system (Brassler, Savannah, GA) and the AEU-20 Endodontic System (Dentsply-Tulsa Dental, Johnson City, TN) electric motor (Figure 4) in a crown down method, starting with a #40/0.06 rotary file (Figure 4). RcPrep (Premier Dental Products, King of Prussia, PA) was used as a lubricant between each rotary file and 2 ml of 6.0-percent NaOCl as an irrigating flush to remove any dentinal debris (Figure 5). The canals were then dried with paper points and divided in the following groups (Figure 6).

GROUP 1: CONTROL GROUP

Following hand-rotary instrumentation and drying, the teeth were flushed with 5 ml of sterile water and dried with paper points and prepared for SEM.

GROUP 2: ULTRASONIC BYPASS SYSTEM
WITH 6.0-PERCENT SODIUM HYPOCHLORITE

Using the Ultrasonic Bypass System, a 30-gauge tip was placed within one millimeter from the working length and twenty teeth were activated with 6.0-percent sodium hypochlorite at 15 ml per minute flow rate for one minute using pump for a total of 15 ml (Figure 7, 8, and 9). The Ultrasonic Bypass System functions by expressing a constant flow of irrigating solutions through an end-vented stainless steel tip. Attached to this tip is a connection, through which irrigating solution flows from a pump. As the tip is ultrasonically activated, irrigating solution is expressed at a steady rate (15 ml/min) and flow, with constant replenishment. There was a final flush with 5 ml of sterile water was performed and teeth were dried with paper points.

GROUP 3: ULTRASONIC BYPASS SYSTEM WITH 17-PERCENT EDTA

Using the Ultrasonic Bypass System, a 30-gauge tip was placed within 1mm from the working length and twenty teeth were activated with 17-percent EDTA at 15 ml per minute flow rate for one minute (Figure 7, 8, and 9) for a total of 15 ml. A final rinse with 5 ml of sterile water was performed and then teeth were dried with paper points.

GROUP 4: ULTRASONIC BYPASS SYSTEM WITH 6.0-PERCENT SODIUM HYPOCHLORITE AND 17-PERCENT EDTA

Using the Ultrasonic Bypass System, a 30-gauge tip was placed within 1mm from the working length and, following the manufacturer's recommendations; twenty teeth were activated with 6.0-percent sodium hypochlorite at 15 ml per minute flow rate for 30 seconds for a total of 7.5 ml. Following the 6.0-percent sodium hypochlorite, a second irrigating solution (17-percent EDTA) was activated in the same manner (Figure 7, 8, and 9) for a total of 7.5 ml. The total combined amount of irrigating solution used was 15 ml. A final rinse with 5 ml of sterile water was performed and then teeth were dried with paper points.

SECTIONING

Vertical grooves were placed on the buccal and lingual surfaces of all the teeth, using carborundum disc at low speed, not penetrating into the canal, as this would introduce unwanted debris (Figure 10). Using a blade and mallet, each tooth was sectioned with a blow of the mallet on the blade (Figure 11). The segment where most of the apex was visible was used for SEM analysis (Figure 12). The chosen segment for each tooth was further divided into three equal parts by grooving the side of the root with a sharp knife, delineating the coronal, middle and apical segments of the segment. Each segment was dried for three weeks in a vacuum-sealed container (Figure 13), sputter-coated with gold-palladium (Fine Coat Ion Sputter Denton Desk 2 model; Lab X, Ontario, Canada), and then mounted on metallic stubs for evaluation with SEM (Figure 14).

MICROSCOPIC EVALUATION

Using the JSM-5310 High Vacuum Scanning Electron Microscope, the apical, middle, and coronal thirds of the sectioned canal were examined (Figure 15). Under 500X to 1000X magnification, the SEM operator directed the central beam through the center of each third of the root canal. Photographs were taken for the following scoring criteria, which has been used in previous studies:

Score 1: Basically a clean root canal with only few or small debris and smear particles (Figure 16).

Score 2: Debris or smear covering 25 percent or less of the root canal wall (Figure17).

Score 3: Debris or smear covering 25 percent or more, but less than 50 percent of the root canal wall (Figure 18).

Score 4: Debris or smear covering more than 50 percent of the root canal wall (Figure 19). [191, 192]

Two blinded endodontists independently scored the sectioned teeth based on the criteria above. To assure the two evaluators were calibrated, twenty examples from a different study were used to increase inter-examiner and intra-examiner reliability. Images from previous studies, which give an example of each score in the four-point scale was available during the scoring of the images, so the evaluators could reference them. Also, the images were randomly assigned a number and placed in a viewing order, so the evaluators did not develop a bias during the scoring of the images. If any discrepancies occurred between examiners, then the two examiners met to come to a

consensus. Following the scoring of the specimens, mean scores were compared between the different irrigating solutions using the Ultrasonic Bypass System.

STATISTICAL METHODS

Intra-examiner repeatability and inter-examiner agreement of the debris removal scores were assessed using two-way contingency tables, percent agreement, and weighted kappa statistics. Using the consensus scores separately for each of the three locations, the four groups were compared for differences in debris removal scores using a Kruskal-Wallis test, which determines if there are any differences among the four groups. If the overall test were significant, Wilcoxon Rank Sum tests was used to compare each pair of groups.

SAMPLE SIZE

With a sample size of 20 teeth per group, the study will have 80-percent to detect a difference of 0.7 between any two groups, assuming two-sided tests with a nonparametric adjustment at a 5-percent significance level. Sample size calculations were performed using PASS (NCSS, Kaysville, UT).

FIGURES AND TABLES

FIGURE 1. Access with #556 bur.

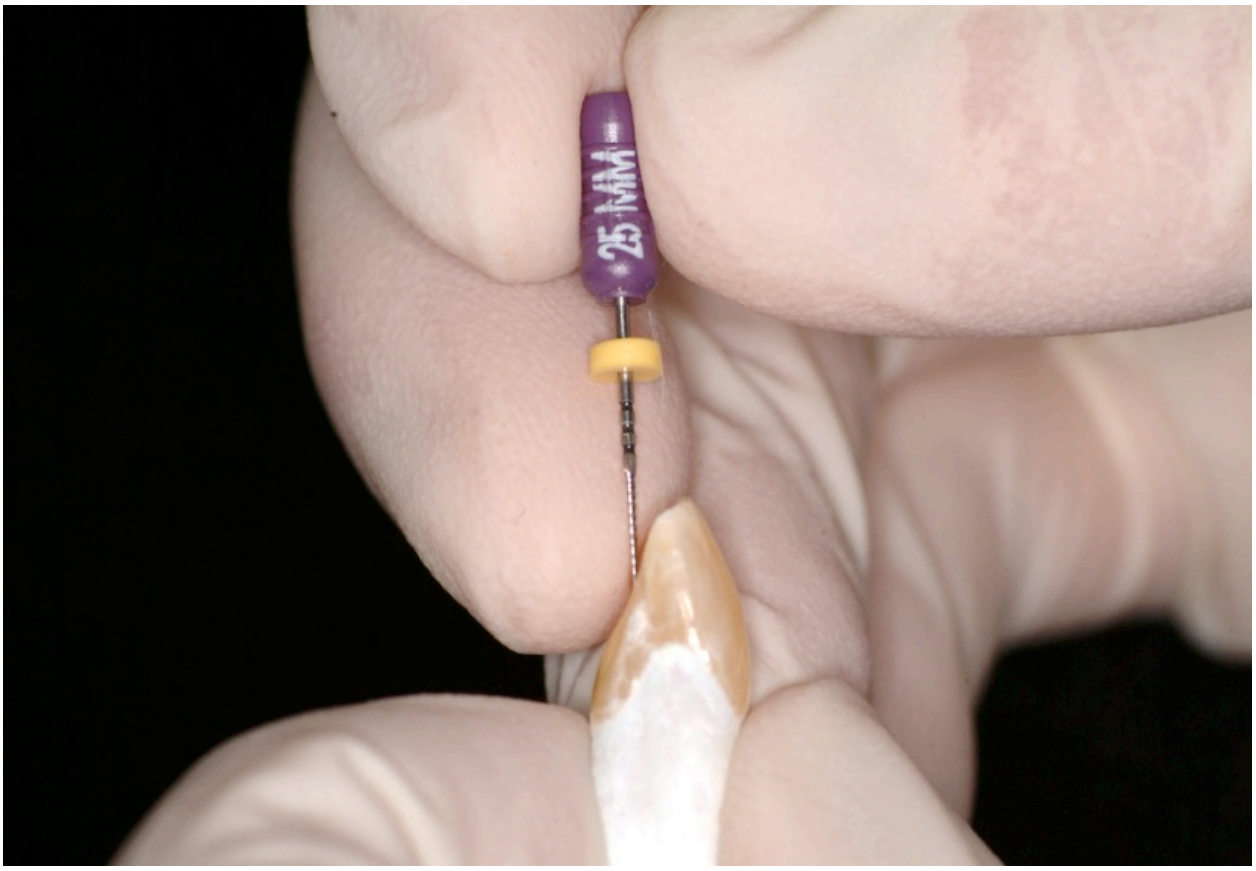

FIGURE 2. Initial file length with #10 hand file.

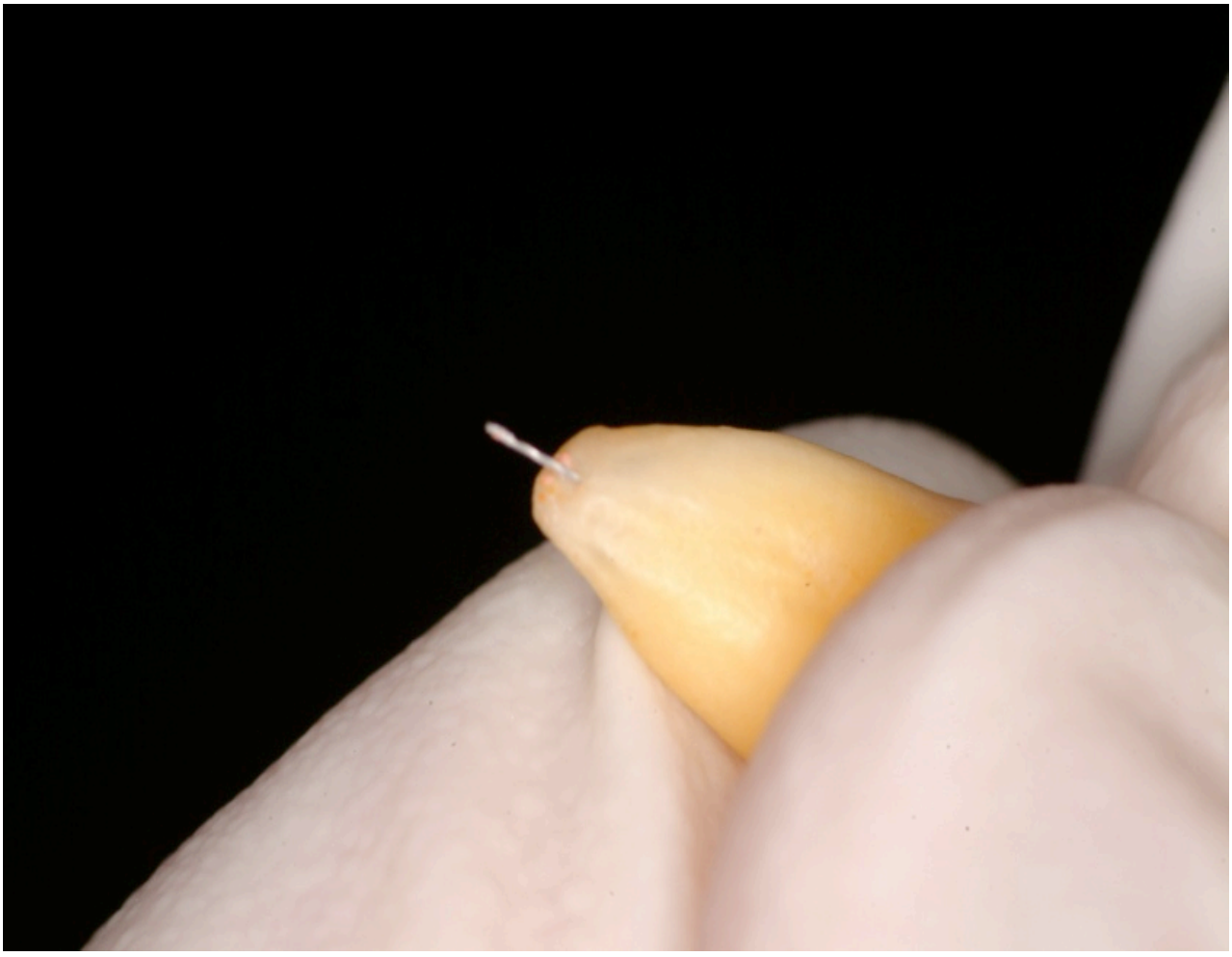

FIGURE 3. One millimeter out apex.

FIGURE 4. EndoSequence™ files.

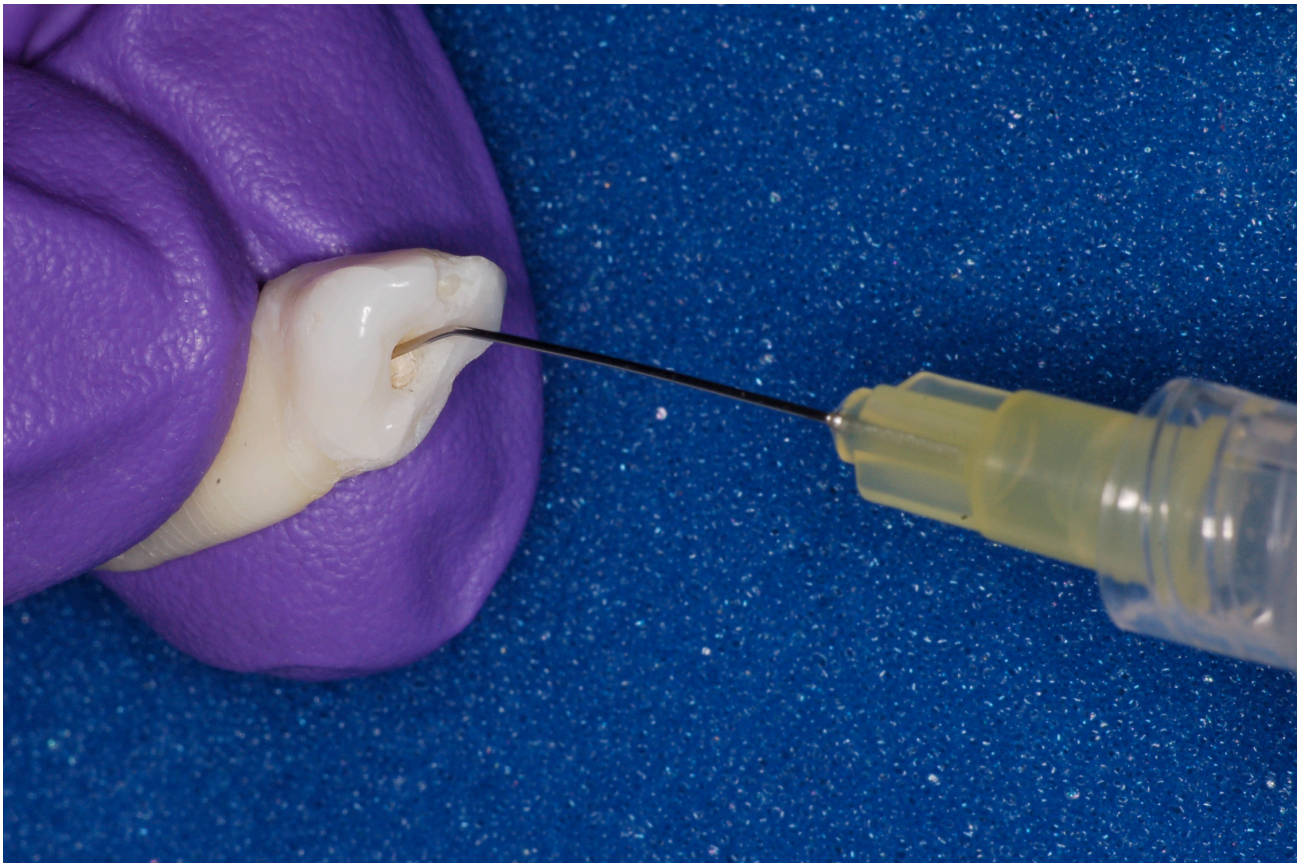

FIGURE 5. Irrigating with 6.0-percent NaOCl.

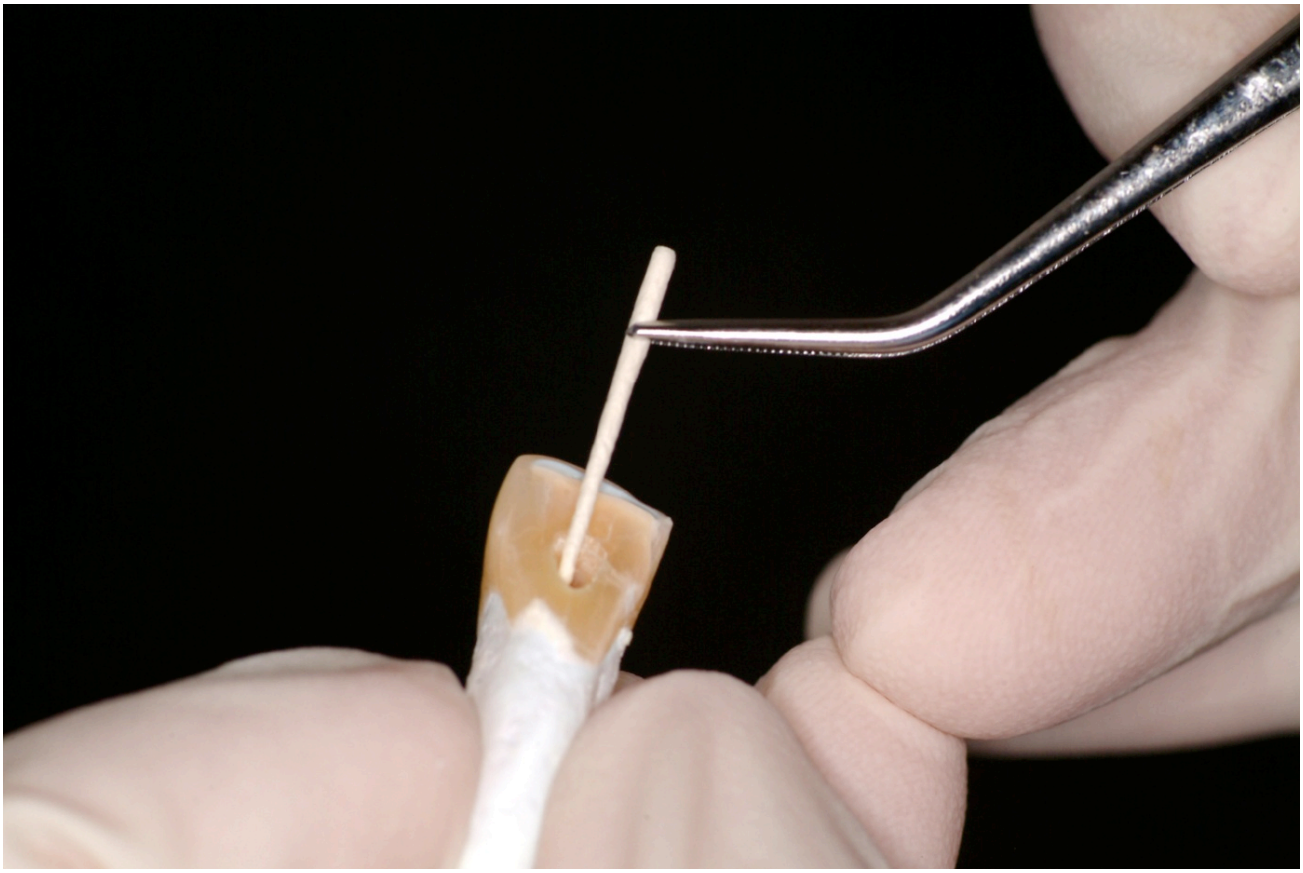

FIGURE 6. Paper point drying.

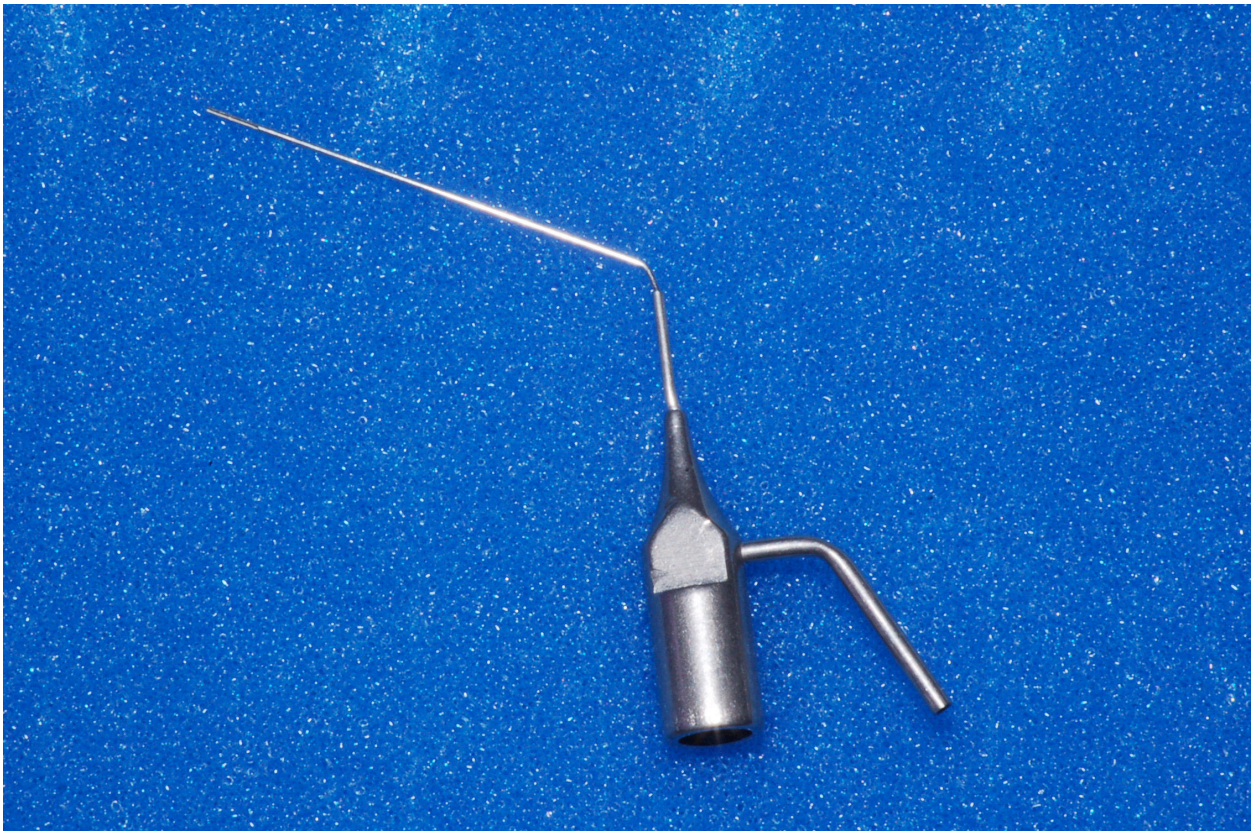

FIGURE 7. Ultrasonic Bypass System™ tip.

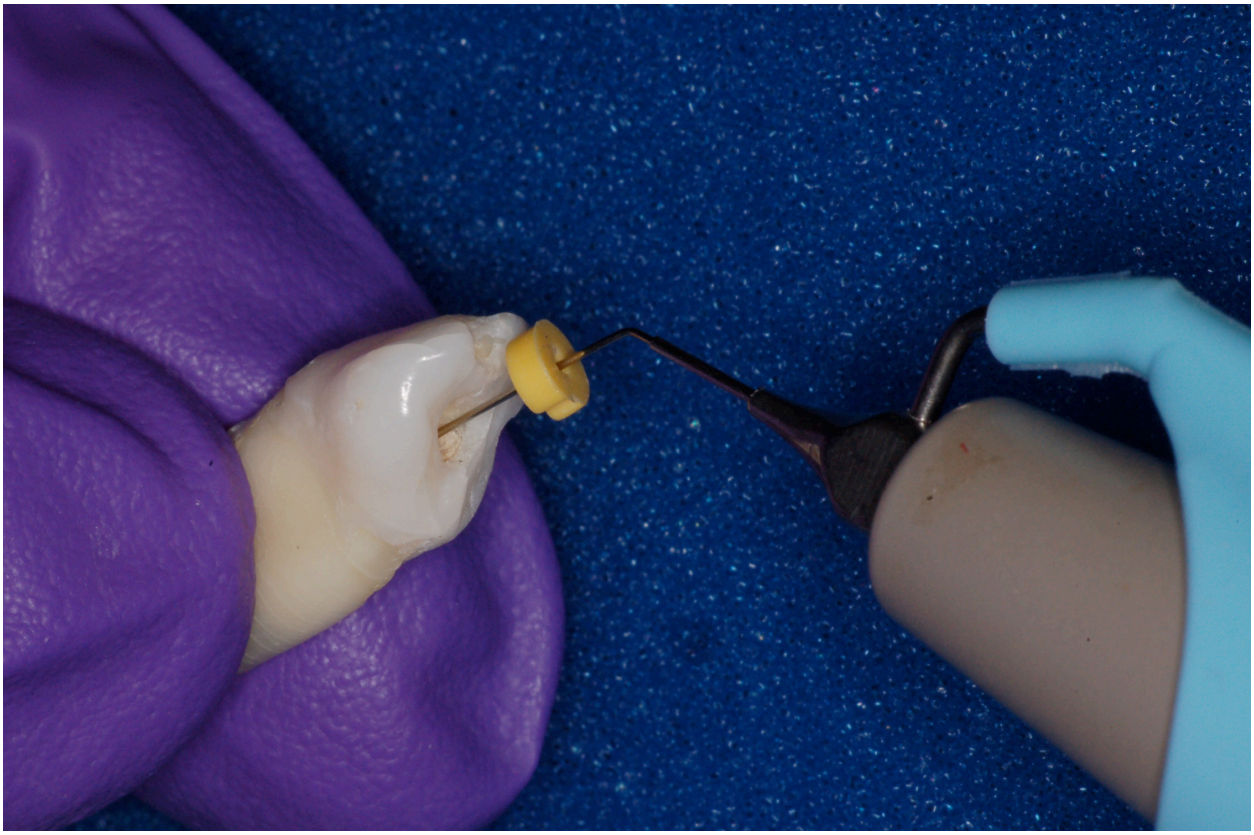

FIGURE 8. PUI with Ultrasonic Bypass System™.

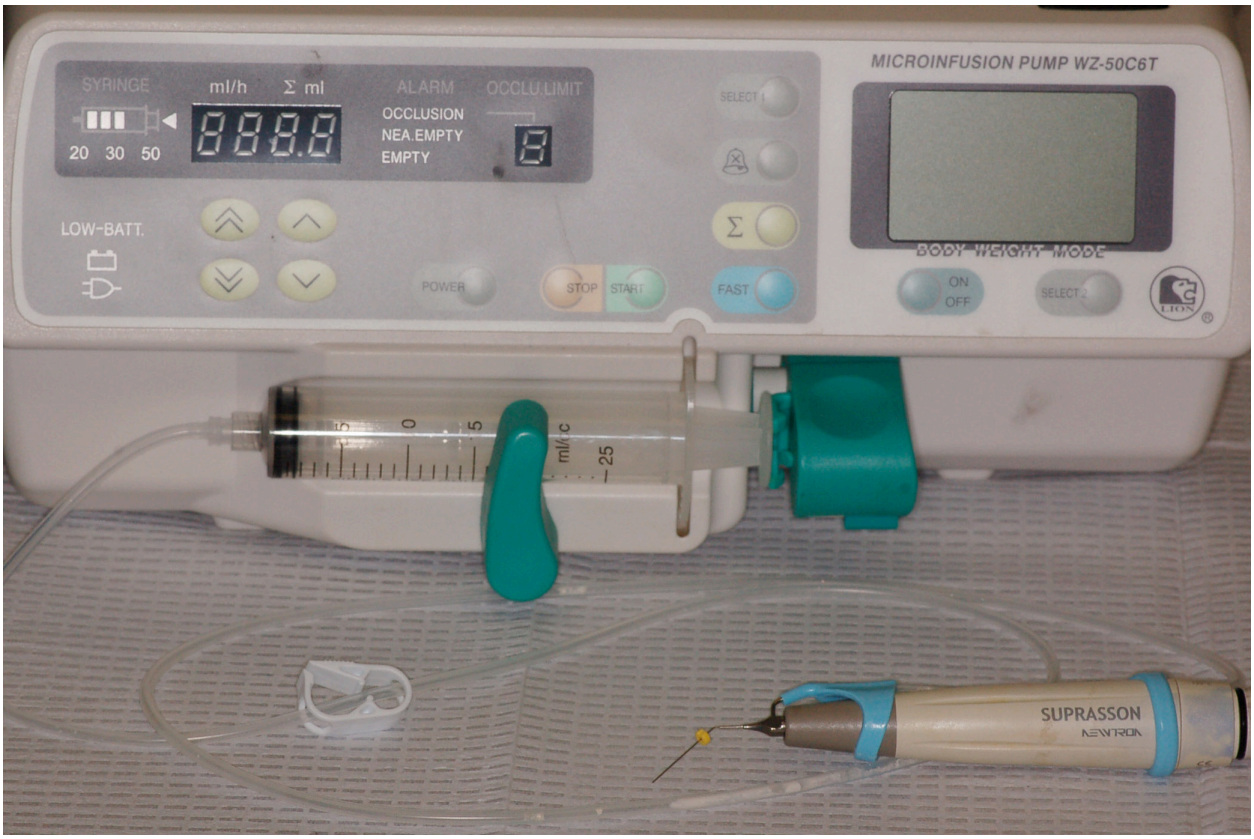

FIGURE 9. Pump for Ultrasonic Bypass System™.

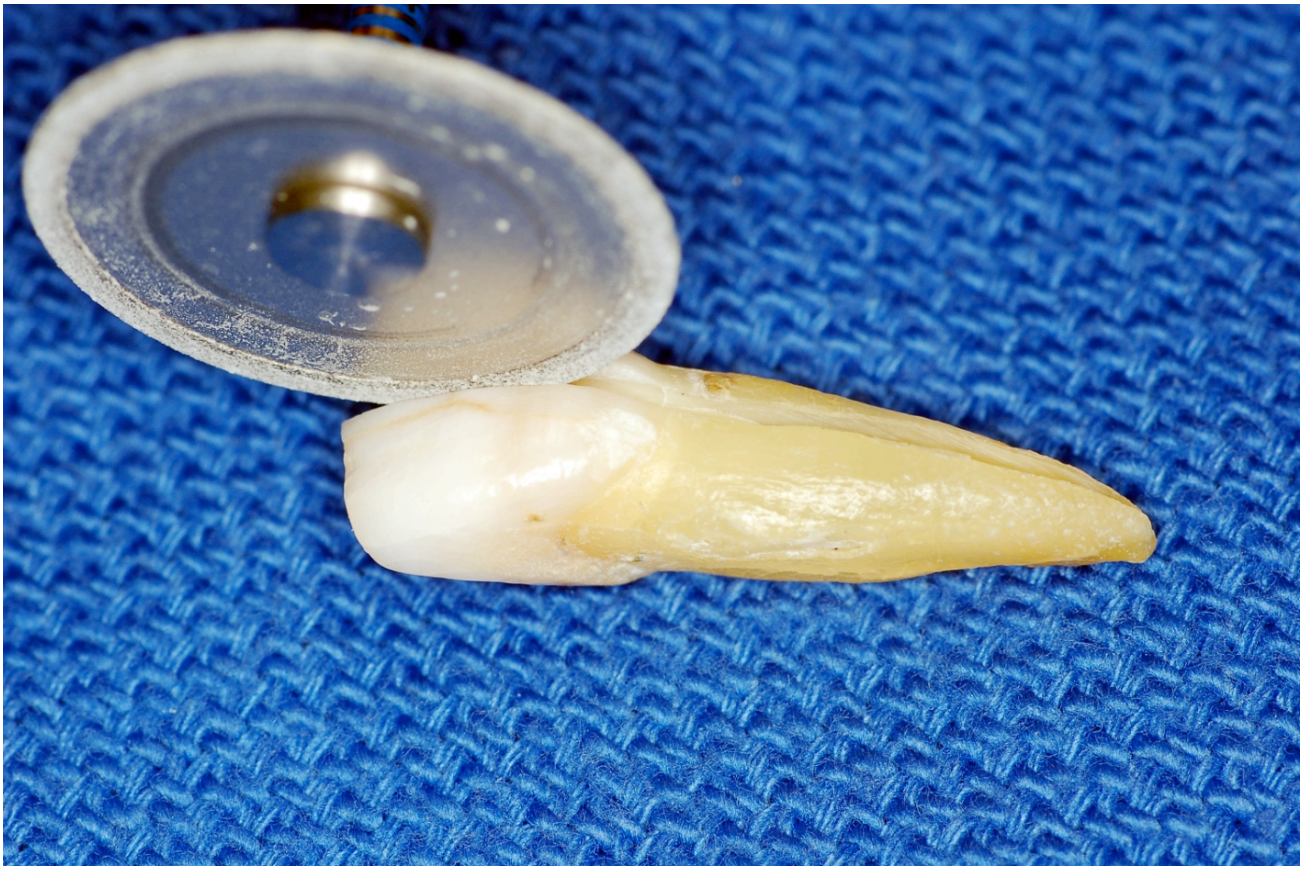

FIGURE 10. Longitudinal groove with caborundum disc.

FIGURE 11. Mallet used to break in sections.

FIGURE 12. Sectioned tooth with apex visible.

FIGURE 13. Vacuum drying of specimens.

FIGURE 14. Specimen sputter-coated and mounted.

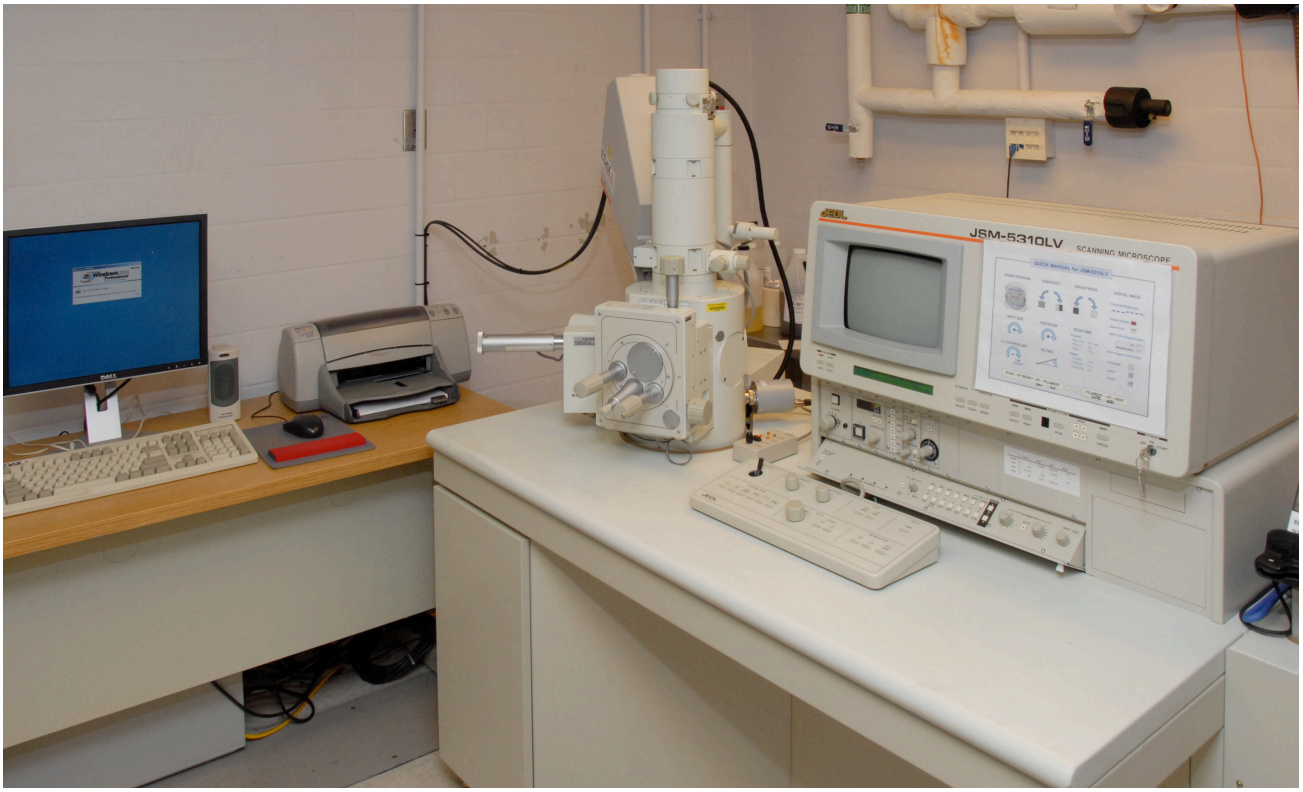

FIGURE 15. Scanning electron microscope.

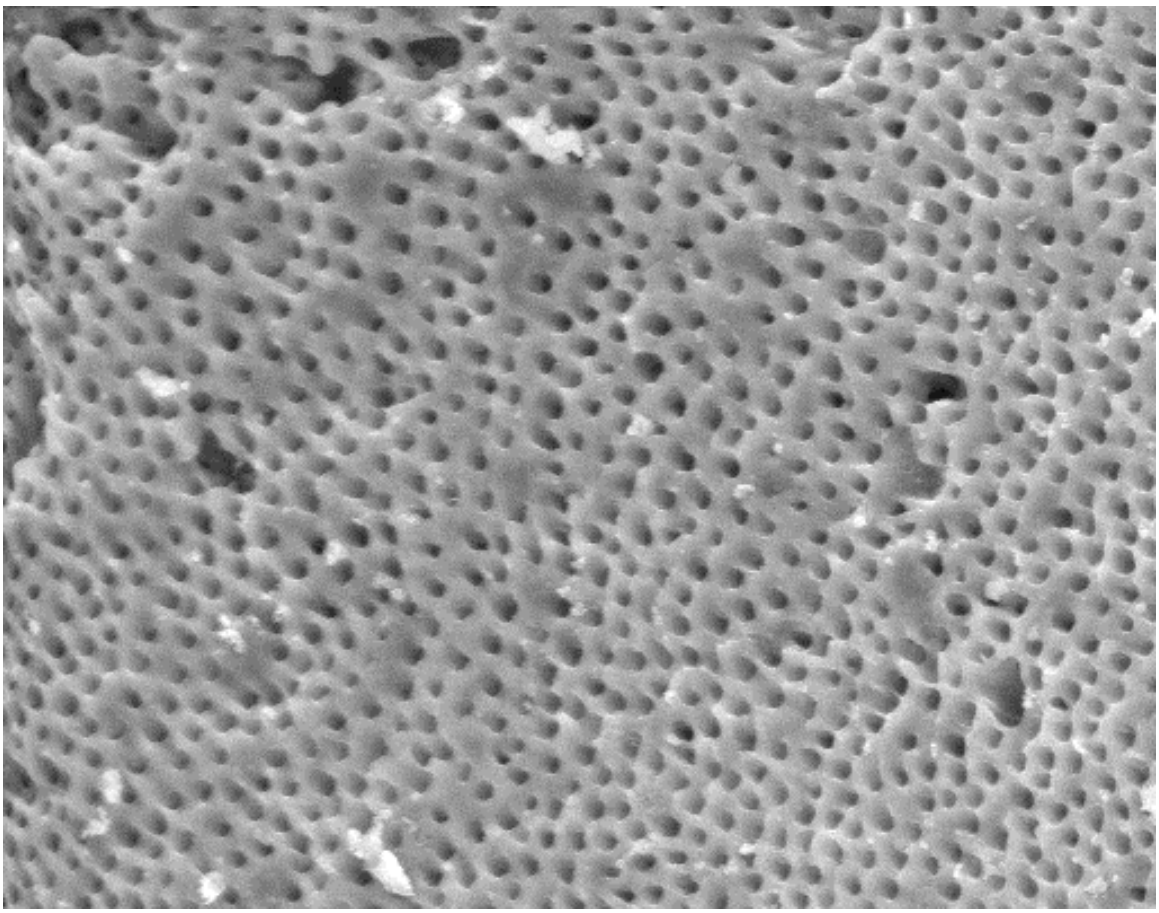

FIGURE 16. Score of one.

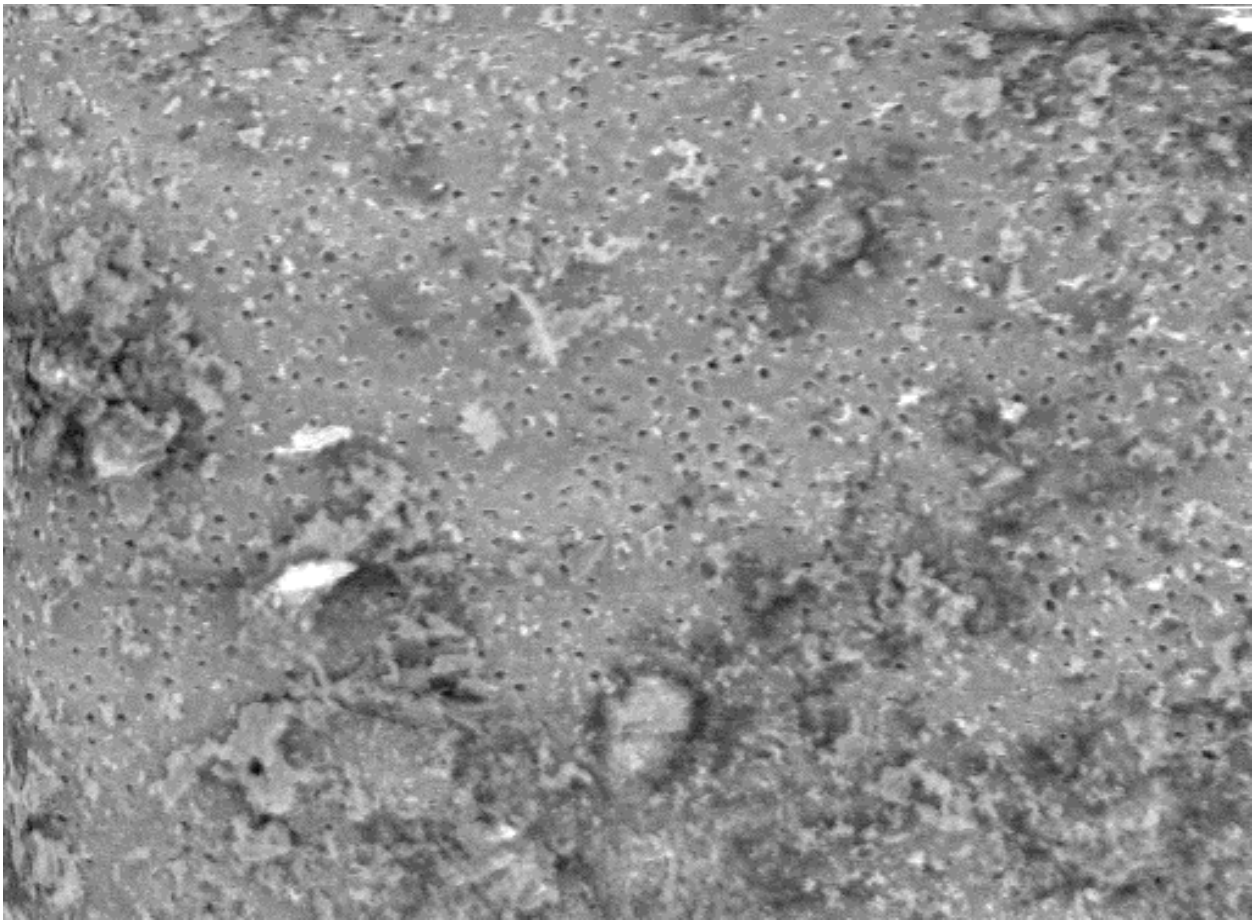

FIGURE 17. Score of 2.

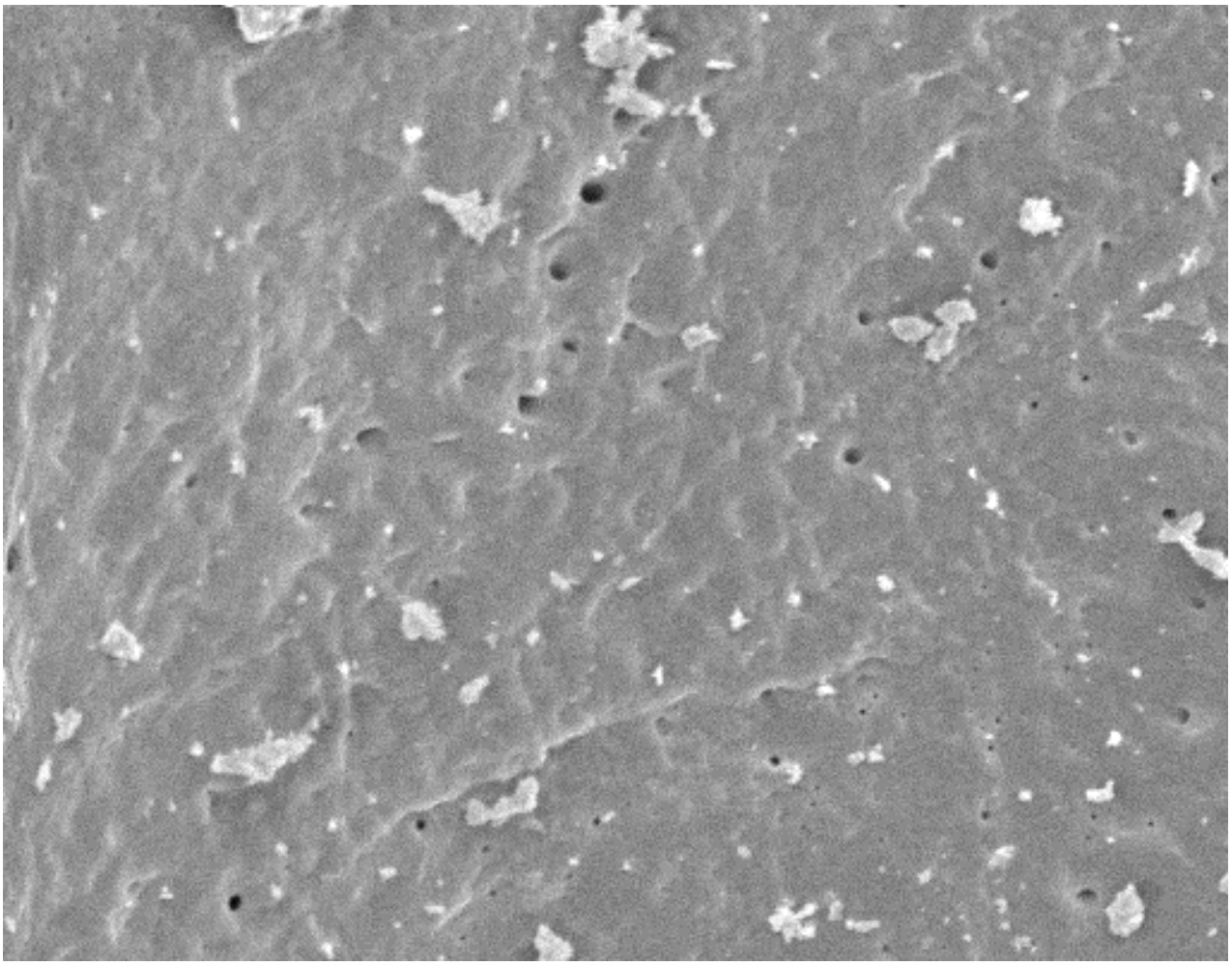

FIGURE 18. Score of 3.

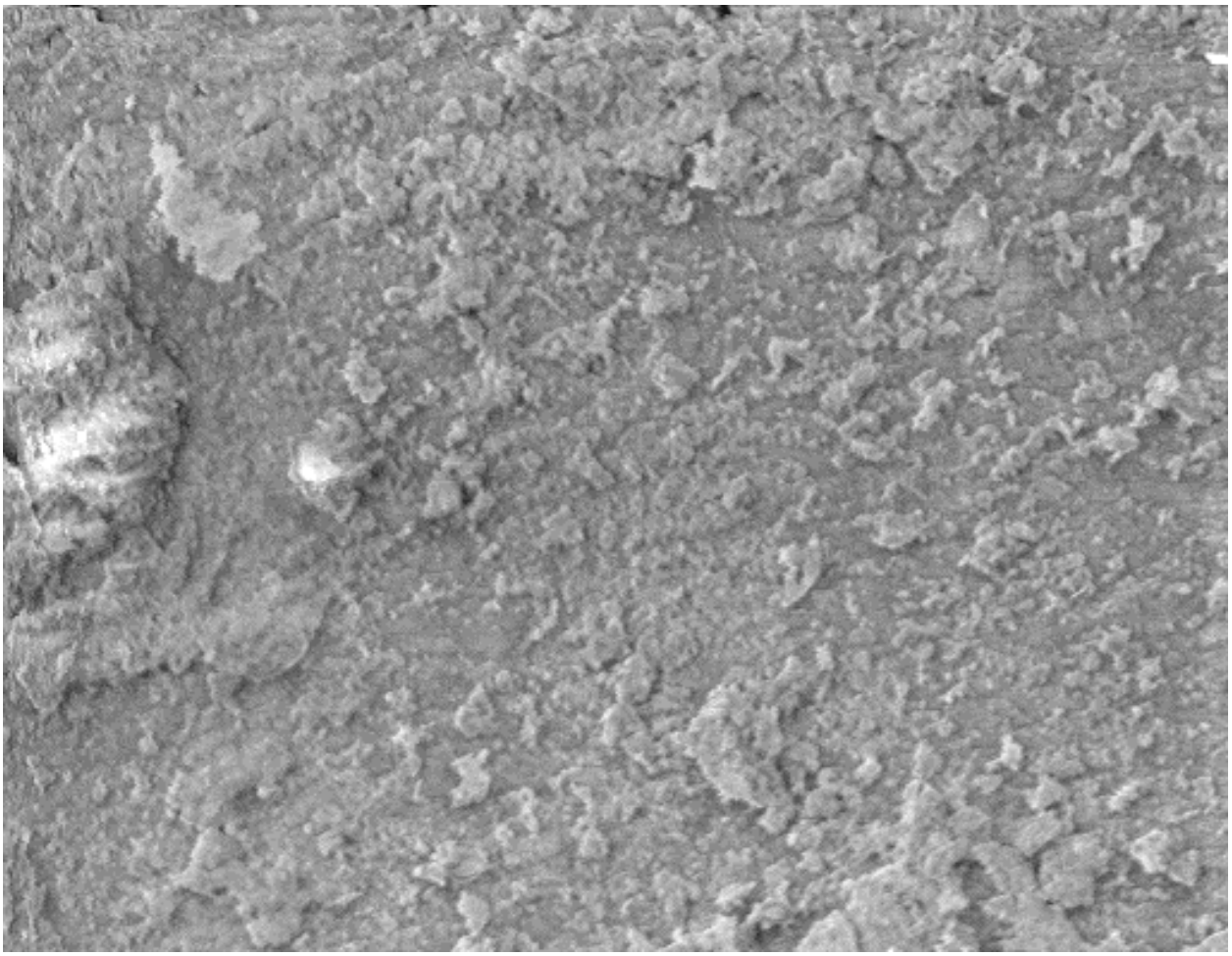

FIGURE 19. Score of 4.

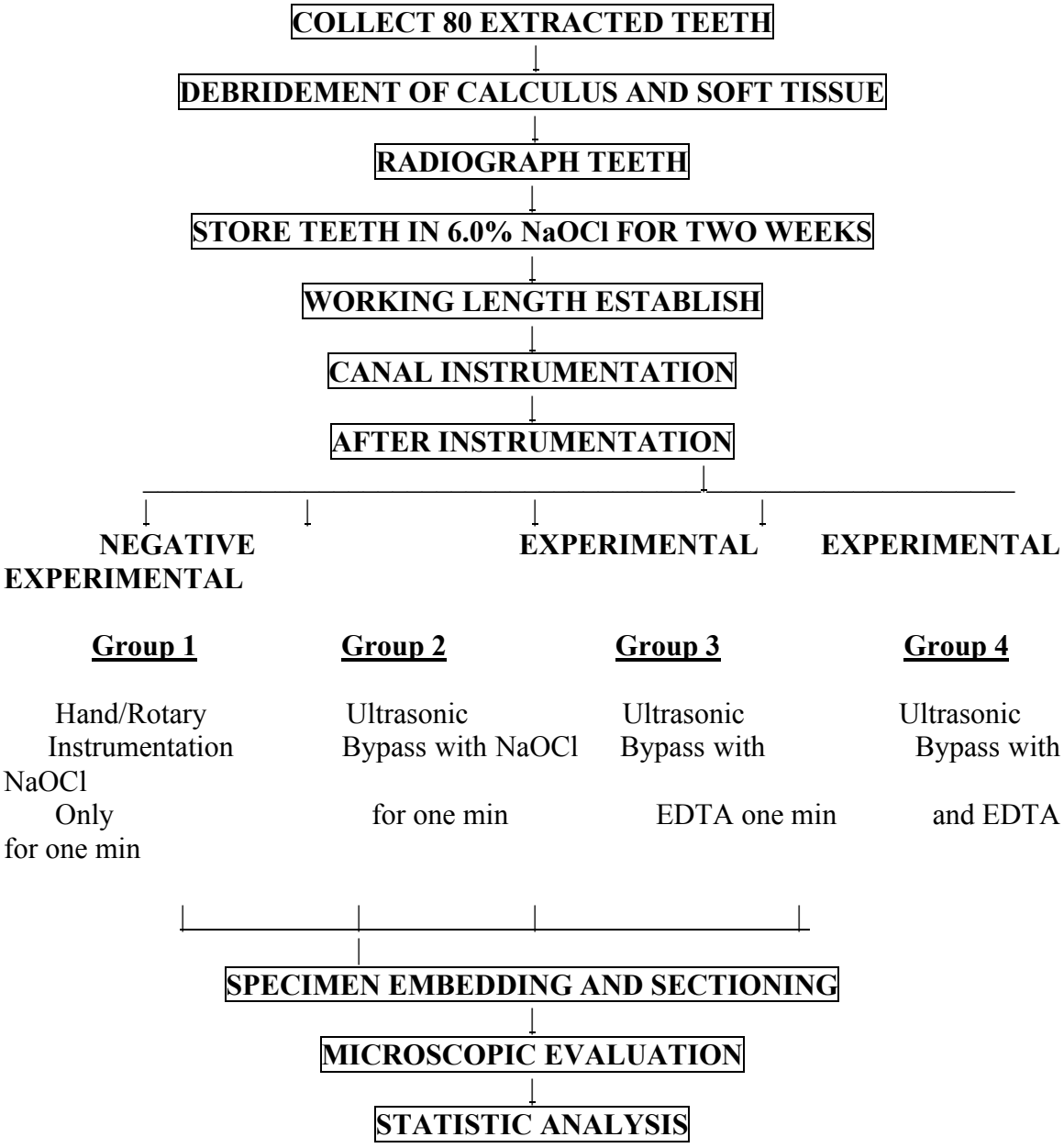

FIGURE 20. Flow chart

TABLE I

Graph comparing groups with locations

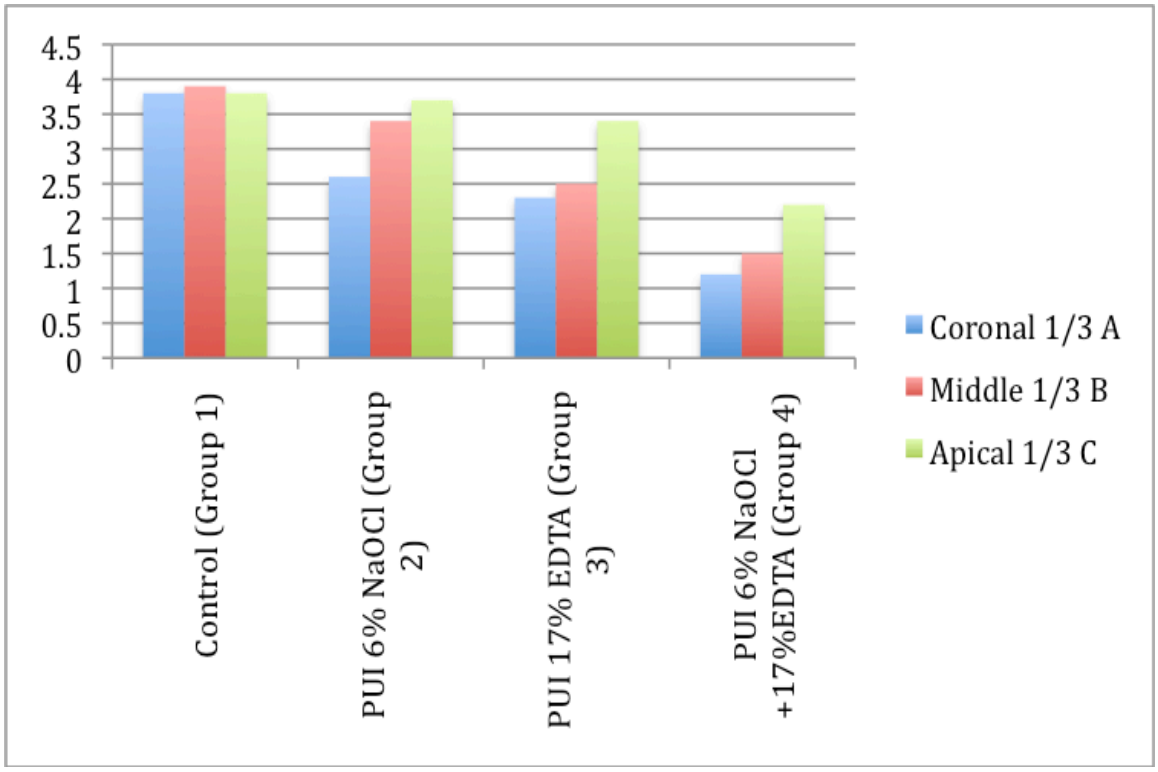

TABLE II

Graph comparing locations among groups

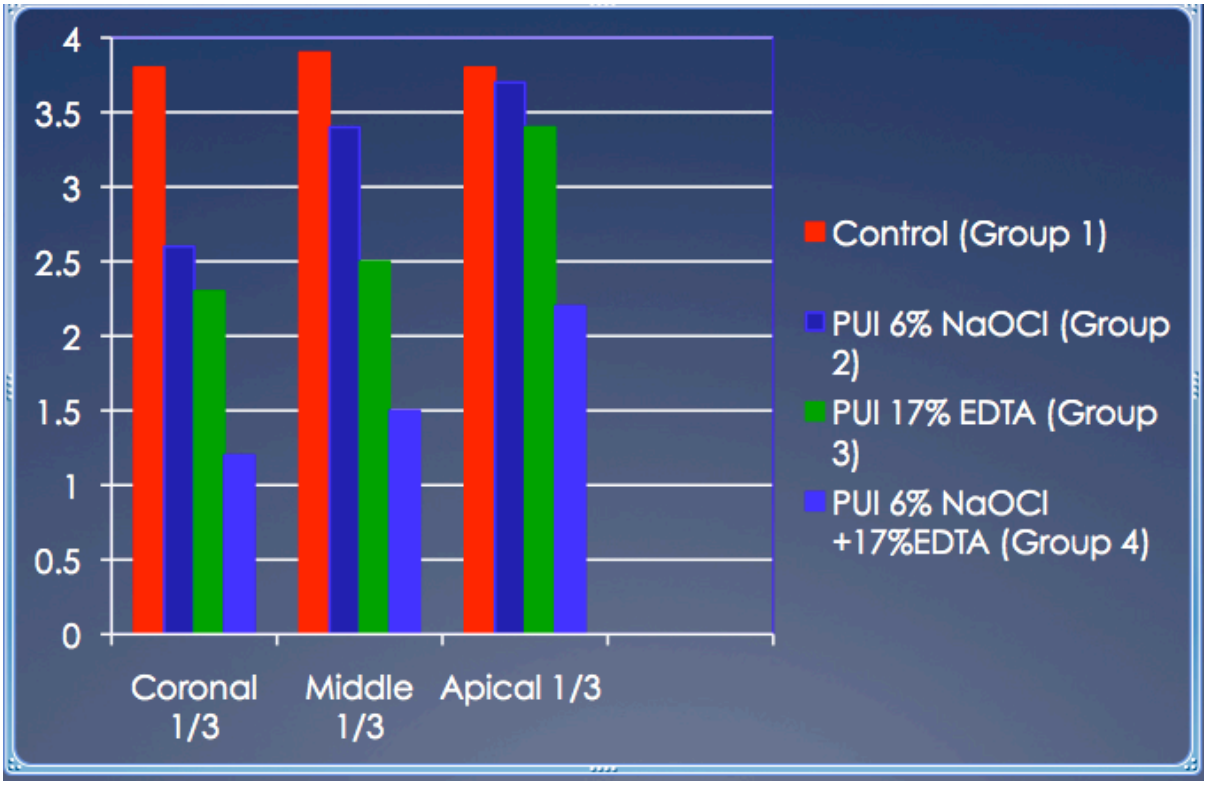

TABLE III

Summary of statistics

	Group	N	Mean	SD	SE
Coronal	Control	20	3.8	0.4	0.1
	NaOCl	20	2.6	1.0	0.2
	EDTA	20	2.3	0.7	0.1
	NaOCl +EDTA	20	1.2	0.4	0.1
Middle	Control	20	3.9	0.4	0.1
	NaOCl	20	3.4	0.8	0.2
	EDTA	20	2.5	0.9	0.2
	NaOCl +EDTA	20	1.5	0.9	0.2
Apical	Control	20	3.8	0.4	0.1
	NaOCl	20	3.7	0.5	0.1
	EDTA	20	3.4	0.9	0.2
	NaOCl +EDTA	20	2.2	0.9	0.2

RESULTS

The results from this study revealed the use of the Ultrasonic Bypass System following hand/rotary instrumentation enhanced debris removal from the canal walls. In particular, the combination of 6.0-percent sodium hypochlorite and 17-percent EDTA when used with the Ultrasonic Bypass System significantly enhanced smear layer removal in all three levels of the tooth compared to all other groups.

Intra-examiner repeatability analysis for examiner one resulted in a weighted kappa = 0.71, with disagreements usually due to a lower score on the repeat evaluation. Intra-examiner repeatability analysis for examiner two resulted in a weighted kappa = 0.60, with disagreements usually due to a higher score on the repeat evaluation. Both of these kappa values are lower than those observed by examiner one, where a similar scoring criterion generated kappa values above 0.80.

Inter-examiner agreement: The inter-examiner agreement analysis showed that disagreements were usually caused by lower scores given by examiner one than by examiner two (weighted kappa = 0.59), with the weighted kappa slightly lower than the intra-examiner kappas as expected.

Group comparisons: For Coronal location there were significant differences in debris scores among groups ($p = <0.0001$), with significantly lower scores for NaOCl+EDTA, NaOCl, and EDTA than Control ($p = <0.0001$, 0.0008, and <0.0001), significantly higher scores for NaOCl and EDTA than NaOCl+EDTA ($p = <0.0001$ and <0.0001), and no significant difference between NaOCl and EDTA ($p = 0.3708$). For

Middle location there were significant differences in debris scores among groups ($p = <0.0001$), with significantly lower scores for NaOCl+EDTA, NaOCl, and EDTA than Control ($p = <0.0001$, 0.0237, and <0.0001), significantly higher scores for NaOCl and EDTA than NaOCl+EDTA ($p = <0.0001$ and 0.0018), and a significantly higher score for NaOCl than EDTA ($p = 0.0028$). For Apical location there were significant differences in debris scores among groups ($p = <0.0001$), with a significantly lower score for NaOCl+EDTA than Control ($p = <0.0001$) and significantly higher scores for NaOCl and EDTA than NaOCl+EDTA ($p = <0.0001$ and 0.0007). There was not a significant difference between either NaOCl or EDTA and Control ($p = 0.5104$ and 0.2619) or between NaOCl than EDTA ($p = 0.6023$).

DISCUSSION

A major challenge in endodontic therapy is cleaning of the root canal system by removal of pulpal tissue, bacteria, and removal of smear layer. Thus the goal should be to eliminate microorganisms within the canal system and eliminate potential substrate, such as pulp tissue, wherein microorganisms may inhabit. Mechanical means, such as hand instrumentation have been the early attempts at accomplishing this goal in endodontic therapy. Mechanical preparation with hand instrumentation alone, however, has not been shown to thoroughly debride a root canal system, especially in the apical third of roots, fins, and isthmuse.[96, 193]

A better more efficient approach to root canal therapy is the incorporation of sodium hypochlorite in chemo-mechanical preparation of the root canal wall. Sodium hypochlorite is a common irrigating solution and shows properties of tissue dissolution and bacteria elimination.[3, 146] However sodium hypochlorite is limited in its properties to remove smear layer and difficulty in reaching all aspects of the root canal system.[194]

The inability to completely remove smear layer further complicates endodontic therapy, especially in light of biofilms found associated with infections of odontogenic origin.[54, 57, 65] Within the smear layer is a possible environment where bacteria can reside and be incorporated into a complex biofilm, unaffected by host defenses and even antimicrobials.[126] This nidus for potential persistence of periapical inflammation and infection is paramount in endodontic therapy and its complete removal remains the primary goal of endodontic therapy. Thus, irrigating protocols must address the goals of

not only microorganism elimination, but ultimately of complete biofilm elimination, which would require the removal of smear layer.

Because of the limitation sodium hypochlorite has in smear layer removal, EDTA has been employed in many irrigating protocols. EDTA functions by removing inorganic components of the smear layer, opening the dentinal tubules, exposing bacteria to the antimicrobial effects of sodium hypochlorite, and ultimately aiding in biofilm elimination.[126] Also to assist the irrigating regimens, passive ultrasonic irrigation has been incorporated to better distribute solutions effectively in the areas of fins, isthmuses, and apical third areas of root canal systems. Studies have shown better debridement of these above-mentioned canal irregularities, as well as better antimicrobial properties with the use of PUI during endodontic therapy.[11, 13]

The results from this study concur with that of Gueisoli et al.[155] The use of the Ultrasonic Bypass System used with the combination of sodium hypochlorite and EDTA, as an irrigating regimen, effectively removed smear layer in the middle and coronal areas of the root canal. Also the results from this study are similar to those found by Cameron et al.,[157] Ciucchi et al.,[162] and Abbot et al.[166, 171, 172] in that the Ultrasonic Bypass System™ could not completely remove smear layer from the apical third. However, when compared to the control of just hand/rotary instrumentation and the irrigating regimens of PUI with sodium hypochlorite alone and PUI with EDTA alone, PUI with sodium hypochlorite followed by EDTA was significantly better at removing smear layer. Although Burleson et al.[11] did not use EDTA in their experimental groups, the results from this study enhance their findings that using the Ultrasonic Bypass System after

hand/rotary instrumentation significantly debrided fins, isthmuses and the apical 1 mm to 3 mm of the canals using the Ultrasonic Bypass System after hand/rotary instrumentation.

With the attention biofilms are drawing in the endodontic literature, future studies evaluating the Ultrasonic Bypass System could focus on not just smear layer removal but actual biofilm removal. Many studies have created *in-vitro* biofilms that were then treated with different irrigating solutions to evaluate biofilm elimination.[125] This same protocol could be researched with the Ultrasonic Bypass System to see its efficacy in biofilm removal.

Another important area that could be further evaluated would be the effect of vapor lock, which is a pocket of air created in the apical portion of a root canal when a needle with irrigating solutions is placed in the canal. This pocket of air prevent irrigating solutions from penetrating the apical extent of the root canal system, thus impeding the effects of the irrigating solutions.[195] In a closed system the tooth is restricted at the apical extent and there is more possibility of a vapor lock occurring. Tay et al.[195] showed that in bench top studies an open system (where there is least chance of vapor lock) showed better smear layer removal when compared to a closed system. In future studies using the Ultrasonic Bypass System, a closed system could be used by enclosing the apical extent of the tooth and to evaluate it ultrasonic activation is able to overcome the vapor lock.

During the use of the Ultrasonic Bypass System in this study, two stainless steel tips separated. Any procedural error such as this introduces challenges during endodontic therapy, so limiting this is important. Recently plastic tips have been created for use with the Ultrasonic Bypass System. A study comparing the stainless steel tips and the plastic

tips would be enlightening. Advantages to the use of plastic tips during ultrasonic activation are: safer to use with less separation and less damage to the canal walls when compared to the stainless steel tips which have higher frequency of separation and more collateral damage to canal walls when contact is made.

SUMMARY AND CONCLUSION

The addition of a one-minute PUI with the Ultrasonic Bypass system significantly enhanced the removal of smear layer when compared to the hand/rotary instrumentation with conventional irrigating solutions. The Ultrasonic Bypass System when used with the combination of 6.0-percent NaOCl and 17-percent EDTA after hand/rotary instrumentation significantly removed smear layer at the coronal, middle, and apical areas of a tooth when compared to the following groups:

- Hand/rotary instrumentation alone.
- Hand/rotary + Ultrasonic Bypass System with 6.0-percent NaOCl.
- Hand/rotary + Ultrasonic Bypass System with 17-percent EDTA.

In the coronal and middle thirds of the tooth, the one minute addition of the Ultrasonic Bypass System with either 6.0-percent NaOCl alone or 17-percent EDTA alone significantly removed more smear debris than the control. There was no significant difference when the Ultrasonic Bypass System was used with NaOCl compared with EDTA, except in the middle third where PUI with EDTA was significantly more effective. In the apical third the combination of NaOCl and EDTA with the Ultrasonic Bypass System was significantly more effective in smear removal than any other group. A one-minute PUI with the Ultrasonic Bypass System combined with NaOCl and EDTA is significantly better in smear removal and ultimately will result cleaner canal walls.

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APPENDIX

APPENDIX I

Debris and smear layer score for Group one (Control group) at each location by two examiners

	First Readings			Second Reading	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
1A	4	4		3	4
1B	4	4		4	4
1C	3	3		4	4
2A	4	4		3	4
2B	3	3		4	4
2C	3	3		4	4
3A	4	4		4	4
3B	3	4	4	3	4
3C	4	4		3	4
4A	4	4		4	4
4B	4	4		4	4
4C	4	4		4	4
5A	4	4		4	4
5B	3	4	4	4	4
5C	4	4		3	4
6A	4	4		4	4
6B	4	4		4	4
6C	4	4		3	4
7A	4	4		4	4
7B	4	4		4	4
7C	4	4		4	4
8A	4	4		4	4
8B	4	4		4	4
8C	4	4		3	4
9A	4	4		3	4
9B	4	4		4	4
9C	4	4		3	4
10A	4	4		4	4
10B	4	4		4	4
10C	4	4		4	4
11A	4	4		4	4
11B	3	4	4	3	4
11C	4	4		4	4
12A	3	3		3	4
12B	4	4		3	4
12C	3	3		3	4
13A	3	3		2	4
13B	4	4		3	4

(continued)

APPENDIX I (cont.)

	First Readings			Second Reading	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
13C	4	4		3	4
14A	4	4		3	4
14B	3	3		2	4
14C	4	4		4	4
15A	3	3		4	4
15B	4	4		4	4
15C	4	4		4	4
16A	4	4		4	4
16B	4	4		2	4
16C	3	3		2	4
17A	4	4		3	4
17B	4	4		3	4
17C	4	4		4	4
18A	3	3		3	4
18B	4	4		2	4
18C	4	4		3	4
19A	3	3		2	4
19B	4	4		4	4
19C	3	3		2	4
20A	4	4		3	4
20B	3	3		3	3
20C	4	4		4	4

APPENDIX II

Debris and smear layer score for Group two (PUI with 6.0-percent NaOCl) at each location by two examiners

	First Readings			Second Readings	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
21A	1	2	2	1	3
21B	2	3	3	2	4
21C	3	3		3	4
22A	3	3		3	4
22B	2	4	4	3	4
22C	4	4		4	4
23A	1	2	2	1	2
23B	3	4	3	3	4
23C	4	4		4	4
24A	2	2		2	4
24B	2	3	3	3	4
24C	3	3		3	1
25A	1	2	1	1	4
25B	3	3		3	4
25C	3	3		4	4
26A	1	2	2	1	2
26B	2	3	3	1	3
26C	1	3	3	1	3
27A	1	3	3	1	3
27B	3	3		2	4
27C	3	3		2	4
28A	2	3	3	2	4
28B	4	4		3	4
28C	4	4		3	4
29A	1	2	2	1	2
29B	1	4	4	2	4
29C	3	4	4	3	4
30A	4	4		2	4
30B	3	3	3	3	4
30C	4	4		4	4
31A	1	2	2	1	3
31B	2	3	3	2	4
31C	2	4	4	4	4
32A	1	2	2	1	2
32B	4	3	4	2	4
32C	1	3	4	1	4
33A	3	3		3	4
33B	1	4	4	1	4
33C	4	4		3	4

(continued)

APPENDIX II (cont.)

	First Readings			Second Reading	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
34A	2	3	4	3	4
34B	2	4	4	4	4
34C	2	3	3	1	4
35A	4	4		3	4
35B	4	4		4	4
35C	1	3	3	1	4
36A	1	1		1	1
36B	1	3	3	1	4
36C	4	4		3	4
37A	1	3	3	4	4
37B	2	4	4	2	4
37C	4	4		4	4
38A	3	4	4	3	4
38B	2	4	4	3	4
38C	4	4		4	4
39A	4	4		3	4
39B	4	3	4	4	4
39C	4	4		4	4
40A	1	2	1	1	4
40B	1	2	1	1	2
40C	4	4		4	4

APPENDIX III

Debris and smear layer score for Group three (PUI with 17-percent EDTA) at each location by two examiners

	First Readings			Second Readings	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
41A	1	1		1	1
41B	1	1		1	1
41C	1	1		2	4
42A	1	2	2	1	3
42B	1	3	2	1	3
42C	3	3		4	4
43A	1	3	2	1	3
43B	3	3		3	4
43C	4	4		4	4
44A	1	3	3	2	4
44B	1	3	2	1	3
44C	2	4	4	2	4
45A	1	3	2	1	3
45B	2	3	2	2	3
45C	4	4		4	4
46A	2	3	3	3	3
46B	2	3	2	2	3
46C	3	3		4	4
47A	4	3	3	3	3
47B	3	3		2	2
47C	4	4		4	4
48A	2	3	2	1	3
48B	3	3		2	3
48C	4	3	4	4	4
49A	1	2	2	2	2
49B	1	2	1	1	1
49C	2	3	3	2	3
50A	1	3		1	3
50B	4	4	4	3	4
50C	4	3	4	4	4
51A	1	4	3	3	4
51B	4	3	3	3	4
51C	4	3	3	3	4
52A	2	3	3	2	3
52B	2	3	2	2	3
52C	4	3	3	2	4
53A	3	3		2	4
53B	4	3	4	4	4
53C	4	3	4	4	4

(continued)

APPENDIX III (cont.)

	First Readings			Second Readings	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
54A	1	2	1	1	3
54B	1	3	3	1	4
54C	3	4	4	3	4
55A	1	3	3	1	3
55B	1	2	2	1	3
55C	2	4	4	2	4
56A	1	2	2	1	3
56B	2	3	3	2	3
56C	4	3	4	3	4
57A	1	3	3	2	3
57B	1	2	2	2	3
57C	1	3	3	1	4
58A	1	2	2	2	3
58B	2	2		2	3
58C	4	3	4	4	4
59A	1	2	2	2	3
59B	1	1		2	2
59C	1	1		2	3
60A	1	2	2	1	3
60B	4	3	4	4	4
60C	3	4	4	4	4

APPENDIX IV

Debris and smear layer score for Group four (PUI with 6.0-percent NaOCl and 17-percent EDTA) at each location by two examiners

	First Readings			Second Readings	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
61A	1	1		1	1
61B	4	3	4	4	4
61C	4	3	3	3	4
62A	2	1	1	2	1
62B	2	1	2	2	2
62C	4	3	4	4	4
63A	1	1		1	2
63B	1	2	1	1	2
63C	4	3	3	2	4
64A	1	1		1	2
64B	1	1		1	1
64C	2	2		2	4
65A	3	2	2	1	3
65B	2	3	3	1	4
65C	1	3	3	2	3
66A	1	3	2	1	3
66B	1	2	3	1	3
66C	1	2	2	1	3
67A	1	1		1	1
67B	1	1		1	1
67C	1	1		1	1
68A	1	1		1	1
68B	1	1		1	2
68C	1	1		1	2
69A	1	1		1	2
69B	1	1		1	2
69C	1	1		1	2
70A	1	1		1	1
70B	1	1		1	1
70C	1	3	3	1	3
71A	1	1		1	1
71B	1	1		1	1
71C	2	2		2	2
72A	1	2		1	1
72B	1	1		1	1
72C	3	3		3	4
73A	1	3	2	1	4
73B	1	1		2	4

(continued)

APPENDIX IV (cont.)

	First Readings			Second Readings	
Specimen #	Observer 1	Observer 2	Consensus	Observer 1	Observer 2
74C	1	1		1	3
75A	1	2		1	1
75B	2	2		2	1
75C	3	3		2	3
76A	1	1		1	1
76B	1	1		1	1
76C	2	2		2	3
77A	1	1		1	1
77B	1	1		1	1
77C	1	3	3	1	3
78A	1	2	1	1	2
78B	1	1		1	1
78C	1	1		2	4
79A	1	1		1	1
79B	1	1		1	1
79C	2	2		2	3
80A	1	1		1	1
80B	1	1		1	2
80C	2	2		2	3

ABSTRACT

AN *IN-VITRO* STUDY EVALUATING THE EFFICACY OF THE ULTRASONIC BYPASS SYSTEM™, USING DIFFERENT INTRACANAL IRRIGATING SOLUTIONS

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This *in-vitro*, prospective, randomized study microscopically compared the debridement efficacy of passive ultrasonic irrigation (PUI) using the Ultrasonic Bypass System and different irrigating protocols. Eighty extracted maxillary anterior teeth were randomly assigned to four groups. Teeth were instrumented using EndoSequence rotary instrument system and treated with passive ultrasonic irrigation with different irrigating regimens for one minute. Group one (control) was treated with hand/rotary instrumentation. Group two was treated with hand/rotary instrumentation followed by a one-minute PUI using the Ultrasonic Bypass System with 6.0-percent NaOCl. Group three was treated with hand/rotary instrumentation followed by a one-minute PUI using the Ultrasonic Bypass System with 17-percent EDTA. Group four was treated with hand/rotary instrumentation followed by a one-minute PUI using the Ultrasonic Bypass System with 30 seconds of 6.0-percent NaOCl and 30 seconds of 17-percent EDTA.

Teeth were sectioned longitudinally and each half was divided into three equal parts from the anatomic apex. The half with the most visible part of the apex was used for SEM evaluation. A scoring system for debris and smear layer removal was used. Statistical analysis was performed using a Kruskal-Wallis test, which determines if there are any differences among the four groups. Following this test, a Wilcoxon Rank Sum test was used to compare each pair of groups. The addition of a one-minute PUI with the Ultrasonic Bypass System significantly enhanced the removal of smear layer when compared with the hand/rotary instrumentation with conventional irrigating solutions. The Ultrasonic Bypass System when used with the combination of 6.0-percent NaOCl and 17-percent EDTA after hand/rotary instrumentation significantly removed smear layer at the coronal, middle, and apical areas of a tooth when compared with all other groups. A one-minute PUI with the Ultrasonic Bypass System combined with NaOCl and EDTA is significantly better in smear removal and ultimately will result cleaner canal wall.

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Professional Organizations and Licensure

American Association of Endodontists
American Dental Association
Colorado Dental Association
Colorado State Board
Western Regional Dental Board